ISSN: 0976-0172
Journal of Bioscience And Technology
www.jbstonline.com

ANTIMICROBIALS: HYDROXYAPATITE VERSUS CLOVE, TUMERIC AND INDIAN GOOSEBERRY

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ABSTRACT
Hydroxyapatite was synthesized by Wet Chemical Technique using calcium hydroxide and ortho-phosphoric acid. Then calcination was done at 800^0C. The synthesized HAP powder was characterized on a macroscopic level by FTIR, XRD, SEM and TEM. It was estimated that the HAP sample prepared was nearly pure. Purity is important here since it has to be implanted in human bodies. The need to produce HAP lies in the fact that it has close similarity to the natural bone and hence can be used widely in medical implants for human bones. Its applicability in chemically modified form amongst other fields can be established by conducting vast research especially in the fields of dental caries filling up and also for denture replacements on the whole.

HAP was assessed to be the best antimicrobial when compared with Clove, Turmeric and Indian Goosebberry. In a mixture, as percent content of HAP increased and that of Clove / Turmeric / Indian Gooseberry decreased, the Zone of Inhibition increased. The present study has revealed the supremacy of HAP as an antimicrobial over Clove, Turmeric and Indian Gooseberry which were assessed for their antimicrobial activity individually as well as in mutual combinations with HAP. It is observed that HAP in pure form had 16-18 mm zone of inhibition and was assessed to have maximum antimicrobial efficacy in the present study. In the comparative ranking of antimicrobial efficacy, it was followed by clove, turmeric and Indian Gooseberry in that sequence. Clove showed 12mm zone of inhibition and Tumeric showed 8 mm zone of inhibition. But Indian Gooseberry had 2 mm zone of inhibition and hence it was found out to be least effective antimicrobial agent in the present study.

KEY WORDS :- Antimicrobial, Hydroxyapatite (HAP), Clove, Tumeric, Indian Gooseberry (Amla), *Escherichia coli (E.coli*), *Staphylococcus aureus* (*S. aureus*), Zone of Inhibition, Blood Agar, MacConkey's Agar .

1. INTRODUCTION

India is one of the largest producers of spices. India grows over 50 out of the 86 spices grown worldwide. As such India being a predominant farming country, spices constitute an important group of agricultural commodities. According to World Health Organization, medicinal plants would be the best source for obtaining a variety of drugs. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harborne and Baxter, 1995).The use of plant extract, with antimicrobial properties, can have great role in the management of microbial infections. The plants that are used for medicinal purpose are Amla (Emblica officinalis), Neem (Azadirachta indica) leaves, Aloe vera leaves, Assam Tea (Camellia sinensis assamica) leaves , Clove (Syzygium aromaticum), Tumeric etc. In primeval India, natural herbs and spices were consumed either in food, or used as medicine in order to retain proper sanitation, health and hygiene, and to increase durability of life. For centuries together, Indian spices have made a noteworthy input both in the food industry and in the health care systems like Ayurveda, Unani system etc. The Vedic literature dated back to 2500 B.C. is the main source of information contributing to the development of Ayurveda. Homeopathic medicinal system has also been using spices as one of the chief ingredients in most of their preparations. Apart from being a major part of the Indian culinary, spices also contribute to the modern allopathic organization of healthcare by providing huge number of medicines or parent compounds. Thus these play a key role in our national financial system.

The substances that can either inhibit the growth of pathogens or kill them but have nil or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. In recent years, antimicrobial properties of remedial plants are being increasingly reported from diverse parts of the globe. Spices have advantageous possessions like anti-inflammatory, analgesic, antimicrobial, antioxidant and antimutagenic actions. An inter-relationship between the health-benefiting properties of spices and their use in food needs to be scientifically re-established.The use of spices in traditional cooking is well known, and the antimicrobial effect of spices, such as turmeric is well documented. Studies on the control of pathogenic bacteria have been fundamentally focused on the use of chemical additives. Recent studies indicate that spices in low doses are beneficial to humans.

A large number of plants are used to combat diverse types of infectious diseases. In an era characterised by increasing consumer choice, self-medication and quest for natural therapy, herbal products are used increasingly as an alternative to drugs and supplements. In particular, extracts from many kinds of oriental spice plants are known to possess antimicrobial effect besides being used for the purpose of food preservation, appetite promotion and medicinal purposes.

The essential oil of several plants shows activity against several bacteria, such as Staphylococcus, Bacillus, *Listeria* and *Klebsiella*. These essential oils also possess inhibitory effect on food spoilaging fungi as well as on industrial yeasts. Today, the exploration of naturally occurring antimicrobials for food preservation receives increasing attention. This is ascribed to the customer awareness of natural food products and a growing concern for microbial resistance towards conventional preservation.

ISSN: 0976-0172
Journal of Bioscience And Technology
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2. MATERIAL and METHODS

HAP was prepared using Calcium Hydroxide & Ortho-Phosphoric Acid by Wet Chemical method. 3.7045 gm of 0.5M Calcium Hydroxide was dissolved in 100 ml of Ammonia solution in a beaker. 2.94 ml of 0.3M Ortho-Phosphoric Acid in 100 ml of distilled water was poured slowly and drops wise through the burette into the beaker. Stirring rate of 800 rpm at 60-80^{0}C and a pH of 10.8 was maintained during the titration.

This reaction took place: - **$10Ca(OH)_2 + 6H_3PO_4$ ----> $Ca10(PO_4)_6(OH)_2 + 18H_2O$.**

The obtained precipitate was allowed to settle for 24 hours. Then washed the prepared chemical with water 7 to 8 times to remove excess ammonia. Funnel along with filter paper was used to remove excess water and to obtain pure HAP which was transferred to the petri- plate. It was then grinded to form a very fine powder and passed through a sieve. Characterisation of HAP was done by Fourier Transform Infra-Red Spectroscopy (FTIR), X Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The result of XRD Analysis confirms the purity sample of the HAP sample synthesized by wet chemical method and analyzed thereof. (Berry, B, 2017).

2.1. ANTIMICROBIAL ACTIVITY

Pellet Preparation: - Uniformly crushed fine HAP Powder was weighed in different contents to make pellets using a dye-punch. Pellets are made in different ratios in pure form as well as by mixing antimicrobials in different proportions as indicated in the table below. Each pellet carried a total content of 900 mg.

HAP Conc. (Percentage)	Antimicrobial substance Conc. (Clove or Amla or Turmeric) (Percentage)
900(100%)	0(0%)
720(80%)	180(20%)
450(50%)	450(50%)
180(20%)	720(80%)
0(0%)	900(100%)

Excipient was also added to assist in the formation of pellets. To ease the formation of pellets 0.5% of polyvinyl alcohol (PVA) or polyethylene glycol (PEG) was added. PVA and PEG also act as polymer or binder. MacConkey's agar was used to study the efficacy of various prepared pellets. Microbes, against which antimicrobial efficacy was tested included Escherichia coli and Staphylococcus aureus. These are the commonest pathogenic bacteria affecting humans. Taking all standard precautions, media were prepared following manufacturer's instructions. Aseptically pouring was done in various petri-plates followed by setting of the media. Respective bacterial suspensions were prepared in normal saline and then inoculated on plates. Three to four pellets were placed on each plate. Antimicrobial testing was done by Disk Diffusion Method (Baur et.al., 1966). Then plates were incubated at 37^{0}C for 24-48 hours and three zones of inhibition in different directions were measured for each pellet and mean zone of inhibition was calculated accurately. Then comparative efficacy of various antimicrobial substances was assessed and results were inferred with appropriate conclusion.

3. RESULTS

3.1. ANTIMICROBIAL ACTIVITY TABLES

Various zones of inhibition have been depicted in the tables below.

Zone of Inhibition values against ***E.coli*** by Pellet (Disk) Diffusion Method on MacConkey's Agar.

Zones of Inhibition with HAP & Clove

Conc of Clove (mg)	Conc of HAP (mg)	Zone of Inhibition (mm)
900	0	11.8
720	180	12.7
450	450	14.0
180	720	15.1
0	900	17.9

Zones of Inhibition with HAP & Tumeric

Conc of Tumeric (mg)	Conc of HAP (mg)	Zone of Inhibition (mm)
900	0	7.8
720	180	10.2
450	450	12.1
180	720	13.8
0	900	17.6

Zones of Inhibition with HAP & Indian gooseberry

Conc of Amla (mg)	Conc of HAP (mg)	Zone of Inhibition (mm)
900	0	1.7
720	180	4.9
450	450	9.1
180	720	12.8
0	900	17.7

ISSN: 0976-0172
Journal of Bioscience And Technology
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Zone of Inhibition values against ***S.aureus*** by Pellet (Disk) Diffusion Method on MacConkey's Agar.

Zones of Inhibition with HAP & Clove

Conc of Clove (mg)	Conc of HAP (mg)	Zone of Inhibition (mm)
900	0	12.2
720	180	13.1
450	450	14.2
180	720	15.4
0	900	18.3

Zones of Inhibition with HAP & Tumeric

Conc of Tumeric (mg)	Conc of HAP (mg)	Zone of Inhibition (mm)
900	0	8.2
720	180	10.6
450	450	12.5
180	720	14.3
0	900	18.4

Zones of Inhibition with HAP & Indian gooseberry

Conc of Amla (mg)	Conc of HAP (mg)	Zone of Inhibition (mm)
900	0	2.3
720	180	5.3
450	450	9.4
180	720	13.1
0	900	18.3

It was observed that HAP produced zone of inhibition of 18 mm diameter in pure form and thus it was found to have maximum antimicrobial efficacy. It was followed by the antimicrobial activity possessed by of clove, turmeric and Indian gooseberry in that sequence in a decreasing manner. In a concentration of 900 mg each; Clove showed 12 mm whereas Turmeric showed 08 mm zone of inhibition in pure form. Indian Gooseberry (Amla) had 2 mm zone of inhibition in pure form and hence it was least effective antimicrobial in the present study. Against Staphylococcus aureus, Turmeric exhibited a zone of 5.67 mm in a concentration of 10% W/W and that of 7 mm in concentration of 5% W/W.(Cheng, Y.et. al.2014); whereas in my study , the zone of inhibition obtained in case of turmeric is about 8.1 mm on an average . It is a little bit higher than that reported in the mentioned study. Alteration may be because of variation in the degree of purity of turmeric powder utilized in the present study. It was observed that with addition of HAP in other pure compounds [amla, clove or turmeric], the anti-microbial activity of the respective pellet tends to increase as evident from the resultant enlarged zone of inhibition. *E. coli* is more pathogenic than *S. aureus*. Hence antimicrobials affected growth of *E.coli* a little lesser extent than that of *S. aureus.* Thus zones of inhibition formed against ***S.aureus*** are comparaitively larger than those produced against ***E. coli.***

4. DISCUSSION

HAP $\{Ca_{10}(PO_4)_6(OH)_2\}$ nanoparticles were synthesized by simple wet chemical precipitation process. Its crystalline structure was analyzed by FTIR, XRD, SEM and TEM. The synthesized HAP was subjected to to action against gram positive and gram negative bacteria to assess its antibacterial activity. Results indicated its excellent broad spectrum antibacterial activity. Additionally a comparision was made between antimicrobial efficiencies of HAP, clove turmeric and Indian gooseberry. HAP has been reported to produce zone of inhibition with a diameter ranging between 16-19 mm in pure form followed by the antimicrobial activities possessed by of clove, turmeric and Indian gooseberry in that sequence in a decreasing manner. (Bhumika, 2017).

The major reason for choosing hydroxyapatite as a biomaterial in our study remains that it is biocompatible and hence suitable for tissue engineering of the bones. As we observe now-a-days, large number of people are seen suffering from various orthopaedic abnormalities. These are many reasons that lead to these defects which either be due to accident or ageing. HAP is being widely

ISSN: 0976-0172
Journal of Bioscience And Technology
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applied in medical field.(Ragab H. S. et al 2014). The biomaterials are being most extensively found so that they can be used as alternative material. Hydroxyapatite, is one of the such biomaterials among the others. The major reason for choosing the hydroxyapatite as a biomaterial is because of its close similarity to the natural bone. (Berry & Singh, 2017).

Antimicrobial substance is one that is capable of inhibiting or killing disease-causing microorganisms.There is a vast list of antibiotics like ampicillin, gentamycin, ciprofloxacin and so on.These antibiotics act on bacteria as well as on some other microbes. Apart from antibiotics there are many herbal antimicrobials. These include various spices and herbs like cloves, cinnamon mustard, black pepper, red pepper, ginger etc. Spices and herbs have been used since ancient era to enhance the flavor and aroma of foods. Early people understood the use of spices and herbs to preserve foods and even learnt their medicinal utility. Scientists also documented the antimicrobial properties of some spices and herbs; and their sub components. Cinnamon, cloves and mustard possess strong antimicrobial efficacy. Whereas coriander and cumin have medium degree of activity; but black pepper, red pepper and ginger are weak antimicrobials. Research studies conducted in the past decade have proved that growth of various food-borne bacteria and fungi can be prevented by cloves, garlic, cinnamon, sage, onion and other spices. For any food, its moisture content along with fat, protein, and salt components, influences the microbial resistance. Spices / herbs help in preserving pickles and eatables.

The normal amount of spices added to foods for flavor cannot completely prevent microbial growth. Antimicrobial effectivity is highly variable. It depends on kind of spice or herb, medium used for testing, and variety of the microorganisms concerned. Addition of spices aids to preserve eatables at low temperature i.e., refrigeration temperature, at which division of microorganisms is at low rate. In another study, silver doped HAP was synthesized by modified sol gel method at a low temperature (100^0C). X-ray diffraction studies showed that the particles were fully crystalline. It exhibited excellent antimicrobial activities against E. coli and S. aureus.(Jadalannagari S. et al 2013).

Clove, whose scientific name is Syzygium aromaticum, is a plant generally developed in Spice Islands, Indonesia, Pemba and Zanzibar. However prior plantation of the plant was in China. Like thyme, it is utilized as a part of the flavoring of food. Its antimicrobial activity is on gram positive and gram negative bacteria and some fungi. The antimicrobial action of clove is due to eugenol, oleic acids and lipids found in its basic oils. For thousands of years clove oil (eugenol) has been used in dentistry. Eugenol has been used topically in dental practice to relieve pain arising from a variety of sources, including pulpits and dentinal hypersensitivity. Interestingly, eugenol exhibits irritant action in addition to its analgesic effect as found in certain studies. (Thosar, N. et. al., 2013).

In a study conducted by Sofia et. al., evaluation of the antimicrobial activity was conducted on six Indian spice extracts i.e., clove, cinnamon, mustard, garlic, ginger and mint. All are in use in traditional medicines. The antimicrobial activity of these was assessed against three potential foodborne pathogens; namely *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus*. Antimicrobial testing was done by paper disc diffusion method, cup method and dilution method. Extracts of clove, cinnamon and mustard showed good inhibitory, while garlic exhibited moderate activity. Ginger and mint possessed minute antibacterial activity.(Sofia P. K. et al 2006). It is being observed that methanol concentrates of spices have high antimicrobial effect on the microbes. These flavors contain high measure of optional metabolites which possesss antimicrobial activity and as such become bio-preservators.

The antimicrobial effectiveness of vanillin, turmeric and curcumin in the form of Dimethyl Sulfoxide (DMSO) solution as well as polymer packaging has been studied. Five kinds of common pathogens and food spoiling bacteria were used. Staphylococcus aureus and Listeria monocytogenes were taken

ISSN: 0976-0172
Journal of Bioscience And Technology
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as gram-positive bacteria whereas Shigella sonnei, Salmonella typhimurium and Escherichia coli represented gram negative bacteria. Turmeric (Haldi) was taken as antimicrobial agent to be incorporated into packaging materials. Turmeric exhibited promising antimicrobial efficacy.

The spices of Zingiberaceae family were evaluated for antimicrobial activity. Their ethanol extracts were taken. The substances included galangal, ginger, turmeric and krachai. The microbes utilized in the study included Staphylococcus aureus and Escherichia coli. It was performed by using an agar disc diffusion assay. Galangal extract exhibited the strongest effect against S. aureus i.e., inhibited it to the maximum. TEM demonstrated that galangal extract damaged outer and inner membranes and also led to cytoplasm coagulation. (Jirawan O. et. al., 2006).

5. CONCLUSION

The biomaterials are being most extensively searched so that these could be used as alternative material in human body implanting. Hydroxyapatite is one of the biomaterials among the others. Additionally, the orthopaedic injuries majorly make the sport persons suffer due to the associated trauma risk. As such hydroxyapatite is mostly used in treating the bone defects. But it is not being used for the dental problems associated with cavities in teeth. Thus, these current studies focus on synthesizing the hydroxyapatite by different methods and to evaluate the nature and properties of the synthesized hydroxyapatite. The various biomaterials have been found and explored. As it has various advantages such as biocompatibility, high porosity and many other. Now our target is to use this material after standard chemical modifications in treating the dental problems as well.

Apart from having excellent biocompatibility, HAP is having good antimicrobial activity. HAP has much better antimicrobial activity when compared with other natural antimicrobials like clove, turmeric and Indian gooseberry. It is potently active against bacteria like *Staph. aureus* and *E. coli.* Both these bacteria are common human pathogens in wide variety of infections. The antimicrobial activity of clove is because of eugenol (a terpenoid), vanillin,eugeniin and casuarji-citin components. Turmeric contains terpenoids i.e., curcumin and turmeric oil as antimicrobials. And Indian gooseberry has phosphatides, tannins, vitamin C and chebulinic acid as antimicrobial substances. (Ahmad & Beg, 2001). Turmeric is a yellow-orange substance that is obtained from the root of the plant Curcuma. Long lasting activity of Turmeric [Curcuma longa] is due to the presence of Curcumin which belongs to class of Terpenoids. It is effective against bacteria. In case of Clove [Syzygium aromaticum], the chemical compound present is eugenol again belonging to Terpenoid class. Concentrates of Amla (Emblica officinalis), Neem (Azadirachta indica) leaves, Aloe vera leaves, Assam Tea (Camellia sinensis assamica) leaves and Clove (Syzygium aromaticum) buds were found to hinder the development of Staphylococcus aureus, Vibrio cholerae and Pseudomonas aeruginosa. Bioactive segments were steady over vast range of pH values and temperatures. (Mehrotra,S.et. al.,2010).

6. REFERENCES

[1]. Ahmad, I., & Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of ethnopharmacology, vol.74 (2), pp.113-123.
[2]. Baur, A.W. Kirby, W.M.M. Scherris, J.C. Turck, M (1966) “Antibiotic susceptibility testing by a standardized single disk method”, Amer. J. Cl. Pathol, vol.45, pp. 493–496.
[3]. Berry,B.(2017). “X-Ray Diffraction: A Tool to Extensively Characterize the Synthesized Hydroxyapatite”. Research Ambition, vol.2 (1), pp.249-254.
[4]. Berry,B., and Singh,A.,(2017). “Hydroxyapatite In Biomedical Field”, Paripex – Indian Journal of Research,vol.6 (4), pp.629-632.
[5]. Bhumika,(2017). “Chemical Synthesis of Hydroxyapatite and Study of its Antimicrobial Property”. Research Inspiration, vol.2 (3), pp.425 -439.

ISSN: 0976-0172
Journal of Bioscience And Technology
www.jbstonline.com

[6]. Cheng, Y. Ge, C. Lodge, J. Santhanam, K.S.V.; and Lu, L. (2014) "Evaluation of Natural Plant Powders with Potential Use in Antimicrobial Packaging Applications." Journal of Applied Packaging Research, vol. 6 (2), pp.28-39.

[7]. Harborne, S.B., and Baxter H., 1995. Phytochemical Dictionary. A Handbook of Bioactive Compounds from Plants. Taylor and Francis, London.

[8]. Jadalannagari, S., Deshmukh, K., Ramanan, S.R. and Kowshik, M. (2014) Antimicrobial activity of hemocompatible silver doped hydroxyapatite nanoparticles synthesized by modified sol–gel technique, Appl Nanosci vol.4, pp. 133-141.

[9]. Jirawan, O., S. Tomoko, G. Piyawan, E. and Griangsak (2006). "Antimicrobial properties and action of galangal (Alpinia galanga Linn.) on Staphylococcus aureus" LWT , vol.39 pp. 1214-1220.

[10]. Mehrotra,S. Srivastava, A.K. and Nandi, S.P .(2010) "Comparative antimicrobial activities of Neem, Amla, Aloe, Assam Tea and Clove extracts against Vibrio cholerae, Staphylococcus aureus and Pseudomonas aeruginosa". Journal of Medicinal Plants Research, vol. 4(18), pp. 2473-2478.

[11]. Nimri, L.F., Meqdam, M.M., Alkofahi, A., 1999. Antibacterial activity of Jordanian medicinal plants. Pharmaceutical Biology, vol. 37 (3), pp. 196–201.

[12]. Ragab, H. S., Ibrahim, F. A., Abdallah, F., Al-Ghamdi, A. A., El-Tantawy, F., Radwan, N., & Yakuphanoglu, F. (2014). Synthesis and in vitro antibacterial properties of hydroxyapatite nanoparticles. IOSR J. Pharm. Biol. Sci, vol.9, pp.77-85.

[13]. Saxena, V.K., Sharma, R.N., 1999. "Antimicrobial activity of essential oil of Lantana aculeata." Fitoterapia, vol. 70 (1), pp. 59–60.

[14]. Sofia, P. K., Prasad, R., & Vijay, V. K. (2006). "Organic farming-Tradition reinvented." Indian Journal of Traditional Knowledge, vol. 5(1), pp.139-142.

[15]. Thosar, N. Basak, S. Bahadure, R. N. and Rajurkar, M. (2013). "Antimicrobial efficacy of five essential oils against oral pathogens: An in vitro study." European Journal of Dentistry, vol. 7(5), pp. 71-77.