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Editorial

It's a fact to laugh when we use a mosquito coil and find mosquitoes buzzing around. It's because they develops resistance against the coil effect. Now a day we are hearing a lot about diseases caused due to Multi-drug resistance viruses/bacterias. Kudos to recent research showing antibiotic azithromycin effectively kills many multidrug-resistant bacteria, a detailed report presented in the news article.

"A warm smile is the universal language of kindness". One of the most widespread problems faced is dental caries mostly occur due to the presence of microbial species in the mouth. Medical advancement has launched several drugs against the same but most of them are not successful in treating it. The reason behind this is the drug resistant shown by several microbial species. Recently, it has been known that plant extracts have been useful in curing the problem of dental caries. These plant extracts are now been used in oral products. Furthermore a plant product Brinjal is one of the most important crops of solanaceous family. Issues on BT Brinjal went viral and were the common headlines on every newspaper some year's back.

Random Amplified Polymorphic DNA (RAPD) analysis done for four local varieties showed several degrees of similarities and differences in the polymorphism. The data obtained by this analysis can be very useful for further crop improvement and cross breeding.

Here by all the students and faculty members are invited to read and contribute to ''QUEST'' to propagate the idea of knowledge gaining by sharing.

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Research News: About 400 words (1 page)
Research Article: About 2000 words (4 pages)

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2) Author's name (s), *Journal name*, **Volume No**, Page No**,** (year).
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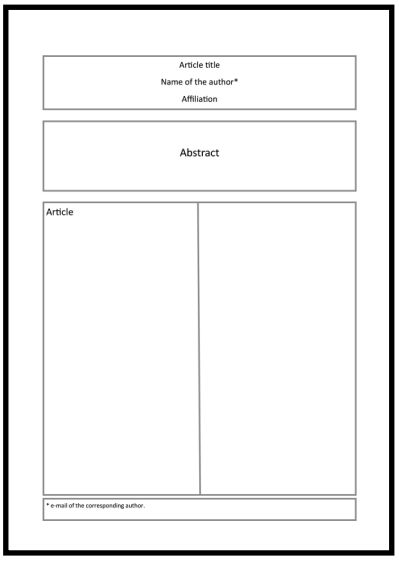

Common antibiotic azithromycin effectively kills many multidrug-resistant bacteria

Contrary to current medical dogma, researchers at University of California, San Diego School of Medicine and Skaggs School of Pharmacy and Pharmaceutical Sciences report that the common antibiotic azithromycin kills many multidrug-resistant bacteria very effectively — when tested under conditions that closely resemble the human body and its natural antimicrobial factors.

Azithromycin is the most often prescribed antibiotic in the United States, where short courses can cure common bacterial infections such as strep throat and sinusitis. But azithromycin, also sold commercially as Zithromax Z-Pak, is never given to patients with some of the most nefarious multidrug-resistant bacterial infections. That's because years of testing in standard laboratory media — the nutrient broth that helps bacteria grow — concluded that azithromycin doesn't kill these types of bacteria.

The bacteria at the center of this study are Gram-negative rods, so-called due to their cell wall structure (they appear "negative" in a classic typing test known as the Gram stain) and their shape. The team studied extremely antibiotic-resistant strains of three medically important Gram-negative rods: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*. These opportunistic pathogens rarely infect healthy people but instead strike debilitated patients in hospitals, such as those with weakened immune systems, or following trauma or surgery, sometimes with deadly consequences. The Centers for Disease Control and World Health Organization have warned that resistance is rapidly spreading in these species, and no new antibiotic candidates are on the horizon.
In this study, team found that simply growing these Gram-negative rod bacteria in mammalian tissue culture media — the same stuff used to sustain human cells in the lab — instead of standard bacteriologic media made a huge difference in their sensitivity to azithromycin. Even more striking, the drug-resistant superbugs were completely wiped out when azithromycin was paired with the antibiotic colistin or with antimicrobial peptides produced naturally by the human body during infection.

To test these promising laboratory results in a live infection system, they moved the experiment into a mouse model of multidrug-resistant *A. baumannii*pneumonia. They treated the mice with a single injected dose of azithromycin at a concentration that mimics the amount typically given by IV to human patients. Twenty-four hours after infection, azithromycin-treated mice had 99 percent fewer bacteria in their lungs than untreated mice. Similarly, in mouse models of multidrug-resistant *P. aeruginosa* and *K. pneumoniae* infections, a single dose of azithromycin reduced bacterial counts by more than 10-fold.

Azithromycin interfere with the protein synthesis and prevents bacteria from growing. It binds to the 50S subunit of the bacterial ribosome, thus inhibiting translation of mRNA. So by considering it as a lead molecule we can do various derivatives of it and design medicines

for other multi drug resistance diseases caused due to bacteria.

Source:
UC San Diego Health System
Published on June 11, 2015

-Contributed by Ravina Sewani, M.Sc IGIBT Sem-VII

Organ-on-a-chip could replace use of animals to test drugs for safety and efficacy

When University of California, Berkeley, bioengineers say they are holding their hearts in the palms of their hands, they are not talking about emotional vulnerability. Instead, the research team led by bioengineering professor Kevin Healy is presenting a network of pulsating cardiac muscle cells housed in an inch-long silicone device that effectively models human heart tissue, and they have demonstrated the viability of this system as a drug-screening tool by testing it with cardiovascular medications.

This organ-on-a-chip, represents a major step forward in the development of accurate, faster methods of testing for drug toxicity. The project is funded through the Tissue Chip for Drug Screening Initiative, an interagency collaboration launched by the National Institutes of Health to develop 3-D human tissue chips that model the structure and function of human organs.

The study authors noted a high failure rate associated with the use of nonhuman animal models to predict human reactions to new drugs. Much of this is due to fundamental differences in biology between species, the researchers explained. For instance, the ion channels through which heart cells conduct electrical currents can vary in both number and type between humans and other animals. The heart cells were derived from human-induced pluripotent stem cells, the adult stem cells that can be coaxed to become many different types of tissue.

The researchers designed their cardiac microphysiological system, or heart-on-a-chip, so that its 3-D structure would be comparable to the geometry and spacing of connective tissue fiber in a human heart. They added the differentiated human heart cells into the loading area, a process that Healy likened to passengers boarding a subway train at rush hour. The system's confined geometry helps align the cells in multiple layers and in a single direction.

Microfluidic channels on either side of the cell area serve as models for blood vessels, mimicking the exchange by diffusion of nutrients and drugs with human tissue. In the future, this setup could also allow researchers to monitor the removal of metabolic waste products from the cells.

This system is not a simple cell culture where tissue is being bathed in a static bath of liquid, instead it is dynamic; it replicates how tissue in our bodies actually gets exposed to nutrients and drugs

Within 24 hours after the heart cells were loaded into the chamber, they began beating

on their own at a normal physiological rate of 55 to 80 beats per minute.

The researchers put the system to the test by monitoring the reaction of the heart cells to four well-known cardiovascular drugs: isoproterenol, E-4031, verapamil and metoprolol. They used changes in the heart tissue's beat rate to gauge the response to the compounds. The baseline beat rate for the heart tissue consistently fell within 55 to 80 beats per minute, a range considered normal for adult humans. They found that the responses after exposure to the drugs were predictable. For example, after half an hour of exposure to isoproterenol, a drug used to treat bradycardia (slow heart rate), the beat rate of the heart tissue increased from 55 to 124 beats per minute.

The researchers noted that their heart-on-a-chip could be adapted to model human genetic diseases or to screen for an individual's reaction to drugs. They are also studying whether the system could be used to model multi-organ interactions. A standard tissue culture plate could potentially feature hundreds of micro physiological .

The engineered heart tissue remained viable and functional over multiple weeks. Given that time, it could be used to test various drugs, Healy said.

This is an incredible chip containing heart tissues on it. This can lead to minimize the tenure of different clinical trial phases of a drug. Also lowers down the ethical issues of testing drugs on humans. We can also try to synthesize a whole organ based on this mechanism. This can be a bench mark of researches in synthesizing organs in-vitro and can be transplant in practice.

Source

University of California - Berkele

-Contributed by , Shirley Dixit,
M.Sc IGMBT Sem-VII

Dental Caries and Medicinal Plant Extracts

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Abstract: Grinding machinery in human affected by microbial species present in the oral cavity and under favourable conditions that lead to dental caries through series of biochemical reactions. The dental caries is a major worldwide problem involving children to adults, though there is a recent advances in the sciences and technologies in dental practice. Inspite of variety of tooth pastes and oral health products, there is still major challenges for the dentist to prevent the dental caries due to antimicrobial drug resistance process. There are many reports on medicinal plants for prevention and cure of many systemic diseases since ancient times. With advancements in science and scientific procedures it is now known that plants have potential for the treatment of serious oral diseases such as dental caries. Currently there is a tremendous usage of plant extracts in the formulation of oral health products particularly tooth pastes and mouth rinse solutions and in the clinical practice.

Introduction

Oral diseases continue to be a major health problem worldwide. The link between oral diseases and the activities of microbial species that form part of the microbiota of the oral cavity is well established. Over 750 species of bacteria inhabit the oral cavity (~50% of which are yet to be identified) and a number of these are implicated in oral diseases. Human oral cavity contains both gram positive and gram negative rods as well as spirochetes. They are distributed on various sites in the human mouth[1]. The gram positive bacteria includes cocci (facultative and anaerobic) & rods (facultative and anaerobic). Gram negative bacteria includes both streptococci (*S. mutans, S. sanguis, S. mitis, S. salivarius and S. mitis*) & staphylococci. The Proportions of each group of bacteria vary at different sites such as plaque, tongue, saliva and gingival region.The bacterial composition also vary with age, for example at the age of 6-9 months in human, oral cavity contains large numbers of *Streptococcus salivarius* (98 % of the total - bacteria). Once teeth is appeared, the *S. mutans* and *S. sanguis* colonized to teeth surface (non-epethelial) and form a dental plaque or biofilm[2]. The oral flora of human may harm their host since some of these bacteria are parasites or opportunistic pathogens. These bacteria also enter in to bone, lung, brain and breast through wounds created by dental manipulation or treatments[3].

Many authors reported activity of plant seeds against cariogenic and other bacteria like *Elettaria cardamomum*[4], *Azadirachta indica*[5], *Quercus infectoria*[6,7] *Viguiera arenaria*[8],*Areca-catechu*[9] and *Punica granatum*[10]. Teeth Colouring, especially teeth blackening with plants, sometimes up to one year, is done for the purpose of preserving the teeth, controlling dental caries and keeping the teeth strong and healthy.

Dental caries

Tooth decay or dental caries is a disease process where *acidic waste products created by*

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oral bacteria cause damage to the *hard* mineralized) tissues of a tooth (enamel, dentin and cementum). If left unchecked, a point can be reached where enough mineral content is finally lost that *a defect (a hole or a "cavity") forms on the tooth's surface.*

The development of dental caries involves acidogenic and aciduric Gram-positive bacteria, primarily the mutans streptococci (*Streptococcus mutans* and *S. sobrinus*), lactobacilli and actinomycetes, which metabolize sucrose to organic acids (mainly lactic acid) that dissolve the calcium phosphate in teeth, causing decalcification and eventual decay. Dental caries is thus a supragingival condition. In contrast, periodontal diseases are subgingival conditions that have been linked to anaerobic Gram-negative bacteria such as *Porphyromonas gingivalis*, *Actinobacillus* sp., *Prevotella* sp. and *Fusobacterium* sp.

Two groups of bacteria are responsible for initiating caries: *Streptococcus mutans* and *Lactobacillus* sp. If left untreated, the disease can lead to pain, tooth loss, infection and death in severe case. Today, caries remains one of the most common diseases throughout the world. The presentation of caries is highly variable; however, the risk factors and stages of development are similar. Initially, it may appear as a small chalky area that may eventually develop into a large cavitation. Sometimes caries may be directly visible; however other methods of detection such as radiographs are used for less visible areas of teeth and to judge the extent of destruction. Caries can be classified by location, etiology, rate of progression, and affected hard tissues. Generally, there are two types of caries when separated by location: caries found on smooth surfaces and caries found in pits and fissures. The location, development, and progression of smooth-surface caries differ from those of pit and fissure caries.

Types of dental caries

Coronal cavities is the most common form in all ages. Coronal cavities are cavities of the visible part of the tooth (crown), usually on chewing surfaces or between teeth. Root caries is more common in older adults as they are more likely to have receding gums that leave part of the tooth root exposed.

Recurrent caries is decay that forms beneath or around existing dental fillings or crowns. Bacteria and food particles can get betwen the tooth and the dental fillings if a filling hasn't been placed properly or if the filling is cracked. Baby bottle tooth decay, a very destructive form of dental caries is common in children who use to fall asleep with a bottle of milk or other sweet liquid in the mouth.

Caries may be acute or chronic, depending on how fast they progress in destroying the enamel. In children and young adults acute decay can create a cavity in a few months while in older adults with chronic caries the process of tooth decay can last for years.

Acid attack a cause of dental caries

Dentists use the term 'acid attack' to summarize the causes of tooth decay. After having a meal, snack or drink, the bacteria of the dental plaque start to convert sugar and carbohydrates of foods into acids. The normal mouth pH of 6.2 to 7.0 starts to drop to acidic values.

If the mouth environment becomes too acidic (pH below 5.5 - 6.0), the acids start to dissolve

the minerals (calcium and phosphate) of the tooth's surface creating microscopic lesions on tooth enamel (demineralization), weakening its structure.

Streptococcus mutans is the most destructive bacterial strain in the mouth as it attaches easily to teeth and produces a lot of acid. Other common but less destructive acid-producing bacteria are lactobacillus and actinomyces.

After all the sugars are consumed by the bacteria, acid production eventually stops and the tooth has a chance to repair itself (demineralization) helped by the minerals of saliva and toothpaste's fluoride.

If dental plaque is not removed regularly, or if sugar is consumed too often, then the demineralization periods are not enough to repair the damage. Eventually a small cavity appears on the tooth enamel. The continuous exposure of the tooth to acids is what causes tooth decay.

Tooth decay can then penetrate through the protective enamel down to the softer, vulnerable dentine and continue to the soft tooth pulp and the sensitive nerves within it.

Although the metabolic activity of plaque bacteria in our mouth is what actually causes dental caries, the underlying causes of tooth decay are in most cases the poor oral hygiene and high sugar consumption.

Pathogenesis of dental caries

Explanation of the destructive process of teeth decay, from the initial stages acid attack up to the total decay of tooth tissues are given in Figure 1A.

The first indication of tooth decay are white spots on the enamel caused by the loss of calcium. Acids have started to dissolve and weaken the tooth enamel (demineralization). At this stage the tooth can rematerialize and fix the weakened area itself with the help of minerals in saliva and fluoride.

If the demineralization process outruns the natural demineralization process, the lesion grows. Over time, the tooth enamel begins to break down beneath the surface while the surface remains intact. Once the decay continues and breaks through the surface of the enamel, the damage is permanent (Figure 1B).

Left untreated, the decay will continue to dentine. When enough of the sub-surface enamel is eaten away, the surface collapses, forming a cavity. The decay must be cleaned out and the cavity filled by a dentist (Figure 1C).

The living part of the tooth, the pulp, becomes damaged. The bacteria invade and infect the pulp of the tooth. The blood vessels and nerves may die due to the infection. Root canal therapy is required to repair the tooth (Figure 1D).

The infection can then spread to form a tooth abscess (collection of pus) around the root tip. As the infection inside the tooth's root canal builds up, the bone around it gets infected. The tooth pain is consistent, especially during the night (Figure 1E).

If the infection is not stopped on time and a root canal therapy is not carried out by the dentist, the tooth might be lost or need to be removed (Figure 1F)

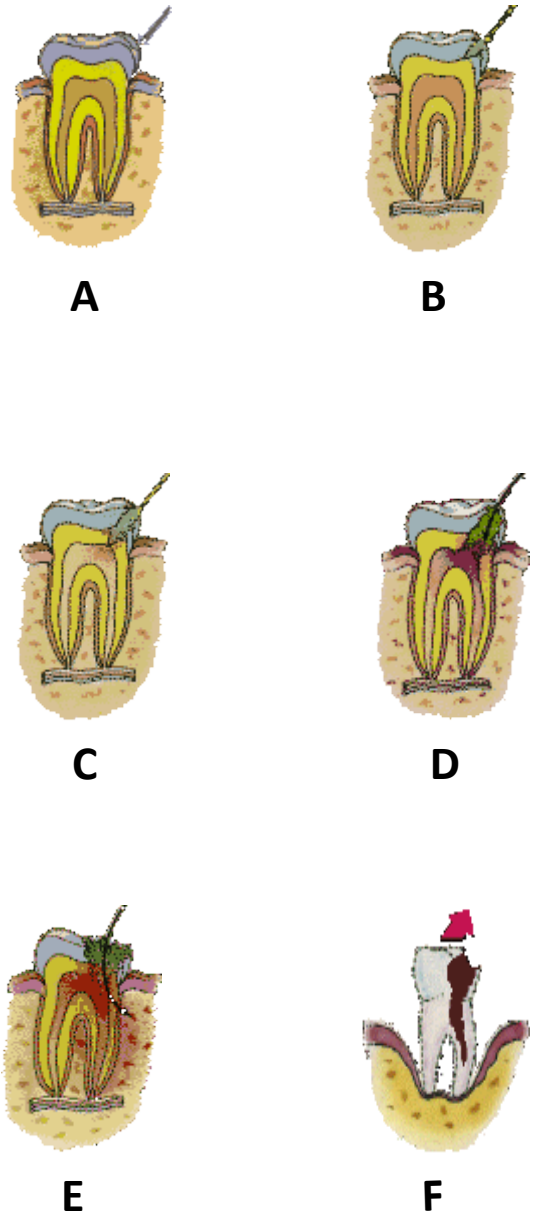

Figure 1 A-F: Various stages during the tooth decaying process

Epidemiology

In Asia and worldwide dental caries is the most prevalent in adult and children. It is the major pathological cause of tooth loss in children[10]. Dental caries affecting all the age groups but the percentage vary. In India, one of two individual experience dental caries.

Prevention

There are five different means by which dental caries can be prevented. They are tooth brush, Oral hygiene, Dietary modification, fluoride therapy and antibiotics. Tooth brush is commonly used to clean the teeth. Personal hygiene care by proper brushing & flossing daily minimizes entry of pathogens. Sugar and other favorable carbohydrates utilized by oral flora produces acids which causes demineralization and ultimately dental caries.

Fluoride supplement helps in prevention of decay by binding with hydroxy appetite crystal in enamel[11]. Antibiotics (Penicillin, ampicillin, erythromycine, methicillin, kanamycin) etc,. Many substances which inhibits adherence such as insoluble glucose from sucrose is also used.

Medicinal Value of Plants

World population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the Contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine.

The drugs are derived either from the whole plant or from different organs, like seed, leaves stem, bark, root, flower, etc,.

drugs are prepared from excretory plant product such as gum, resins and latex. Even the allopathic system of medicine has adopted a number of plant-derived drugs. Medicinal principles are present in different parts of the plant and these medicinal principles are separated by different processes, the most common being extraction.

Plant produce many economically important compounds known as secondary metabolites: saponins, flavonoids, alkaloids, tannins, oxalates, phytates, trypsin (protease) inhibitors, phytohaemaglutinins (lectins) etc,**.** Saponins and flavonoids, have found wide applications in the fields of medicine, pharmacy and food industries as pharmacologically active principles[14], in food, drink and beverage industries as foaming agents[15,16] as antioxidants, preservatives and flavouring agents [17]and in agriculture[18].

Plant as dental drug

About 10 different oral/dental conditions treatable with plants are common in traditional health practice namely: dental caries, toothache, gingivitis, ulcerative gingivitis, mouth ulcers, swollen tonsil, oral thrush, tonsillitis and black tongue[19]. Most common plants in the field include *Piper guineense*, *Xylopia aethiopica*, *Citrus aurantifolia* and *Aframomum melegueta*. For ordinary oral hygiene, teeth are cleaned in the morning by chewing the roots or thin stems of certain plants until they acquire brush-like ends[20]. The antimicrobial activities of the individual chewing sticks have been investigated, showing that all of them were active against the oral microbial flora in varying degrees. The antimicrobial action of *Zanthoxylun zanthoxyloides* is attributed to the presence of benzoic acid derivatives[21]. The phenolic acids were active at a pH of about 5 and the alkaloids (Canthin, berberine and chelerythrine) were active at a pH of 7.5, meaning that the root contained antimicrobial compounds, active at both alkaline pH (during heavy tooth decay) as well as acid pH (after a drink of lime or grape juice). Tella[22] reported antimicrobial properties of the crude root of *Vernonia amygdalina* in gingivitis and toothache.

There are many reports on plants with antibacterial activity like *Elettaria cardamomum*[4], *Azadirachta indica*[5], *Quercus infectoria*[6,7], *Viguiera arenaria*[8], *Areca catechu*[9] and *Punica granatum*[10]. Teeth Colouring, especially teeth blackening with plants, sometimes up to one year, is done for the purpose of preserving the teeth, controlling dental caries and keeping the teeth strong and healthy.

Medicinal plants for dental caries

The global need for alternative prevention and treatment options and products for oral diseases that are safe, effective and economical comes from the rise in disease incidence (particularly in developing countries), increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics. Despite several agents being commercially available, these chemicals can alter oral microbiota and have undesirable side-effects such as vomiting, diarrhea and tooth staining. For example, bacterial resistance to most of the antibiotics commonly used to treat oral infections are penicillins and cephalosporins , erythromycin, tetracycline and derivatives and metronidazole. Other antibacterial agents used in the prevention and treatment of oral diseases—

icluding cetylpyridinium chloride, chlorhexidine, amine fluorides or products containing such agents, are reported to exhibit toxicity, cause staining of teeth or in the case of ethanol (commonly found in mouthwashes) have been linked to oral cancer. Hence, the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals.

The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals. With respect to diseases caused by microorganisms, the increasing resistance in many common pathogens to currently used therapeutic agents, such as antibiotics and antiviral agents, has led to renewed interest in the discovery of novel anti-infective compounds. In particular, traditional medicinal plant extracts or phytochemicals that have been shown to inhibit the growth of oral pathogens, reduce the development of dental plaque, influence the adhesion of bacteria to surfaces and reduce the symptoms of oral diseases.

A number of studies have investigated the activity of plant extracts and products against specific oral pathogens, while others have focused on the ability of the products to inhibit the formation of dental biofilms by reducing the adhesion of microbial pathogens to the tooth surface, which is a primary event in the formation of dental plaque and the progression to tooth decay and periodontal diseases.

Plant extracts and phytochemicals have been demonstrated to inhibit any or all of these stages i.e. cidal activity against cariogenic bacteria, inhibition of adherence/ aggregation tion/biofilm formation and inhibition of glycolytic acid production.

In vitro experiments showed that cacao bean husk extract markedly reduced the growth rate (69–72% reduction) and inhibited insoluble glucan synthesis of *S. mutans* and sucrose-dependent adhesion of *S. mutans* and *S. sobrinus* to a glass surface (85% inhibition at >5 mg ml^{-1})[24].

An ethanol extract of *Helichrysum italicum* (Compositae) powdered flowering tops was found to exert antimicrobial activity against *S. mutans*, *S. sanguis* and *S. sobrinus*[25]. At sub-MIC levels (<31.25 μg ml^{-1}), the extracts were able to reduce cell surface hydrophobicity, adherence to glass and cellular aggregation of *S. mutans* in the presence of dextran.

Conclusion

Before onset of synthetic era, man was completely dependent on medicinal plants for prevention and treatment of diseases. It is already known that the plants contain active principles responsible for the treatment of various ailments. In recent years plant extracts have great potential as antimicrobial compound against variety of pathogens, that can be used to treat infectious diseases. There are many reports suggesting that the plants extracts and purified phytochemicals have potential to be developed into agents which can be used for the prevention and or treatment for oral diseases such as dental caries.

References

1. Hamada S and Slade H. *Microbiol*. **44**,545-638, (1980).
2.Takahashi N. *International Congress Series*; **1284**, 103-112, (2005).
3.Meyer D. Oral pathogen: *from dental plaque to cardiac diseases Microbiology*; 88-95, (1998).
4.Karthy E, Ranjitha P and Mohankumar A. *International Journal of Biology;* **1 (1)**,34-40, (2009).
5.Prashant G, Chandu G, Murulikrishna K and Shafiulla M. *Indian J Dent Res*; 18 **(4)**,148-51, (2007).
6.Adel K and Muhammed S. *Current Research Journal of Biological Science*; **2 (5)**, 333-337, (2010).
7.Muskhazil M., Nurhafiza Y., Azwady N and Dalilah N. J. *Boil. Sci*; **8**,634-638, (2008).
8.Thiago S, Rander R, Niege A, Tatiane C, Carlos H, Martins G, Rodrigo C, Veneziani, F Da Costa, Adriana H, Wilson R, Vladimir C and Sergio R. . *Molecules*; **14**, 191-199, (2009).
9.Joseph I, Ranjit S. *Ethnobotanical Leaflets*; **12**,995-1002, (2008).
10.Jang G, Young S, You C, Obiang O, Min S, Dong H, Hun S, Hyun P, Dong W, Jung R and Dong Y. doi:10.1093/ecam/nep105, (2011)
11.World health organization. Worldwide day: Oral health, 2, (2001).
12.Appelbaum B, Golub E, Holt S, Rosan B. *Infection & immunity*; **25**, 717-728, (1979).
13.Schopke T, Hiller K. Triterpenoid saponins, Part 6. *Die Pharamazie*; **45**, 313-342, (1990).
14.Fenwick G, Heaney R, Mullin W. *Food. Sci. Nutri*; **18**, 123-201, (1983)
15.Oakenfull D and Sidhu G. Saponins. *CRC Press. Inc. Florida* 4,97, (1989).
16.Oleszec W, Price K, Colquhoun I, Jurzysta M, Ploszynski M, Fenwick G. J. *Agric. Food.Chem*; **38**, 1810-1817, 1990).
17.You J, Wang X, Yan Y, Jin F, Huang B. *Chem. Abs.*; **120(7)**, 70, (1993).
18. Waller G, Jurzysta M, Thorne Z. *Bot. Bull. Acad. Sci*; **34**, 1-11, (1993).
19.Hollist N. A Collection of Traditional Yoruba Oral and Dental Medicaments. Edited by the Book Binders and printed by Olubena Printers, Ibadan. Nigeria, (2004).
20.Elujoba A. Medicinal properties of plants with Oral Health Implications. Proceedings of the 2nd Dr. David Barmes'Memorial Public Health Symposium, 25th March 2003, organized by the Regional Centre for Oral Health Research & Training for Africa. JOS in collaboration with WHO Regional Office, Brazzaville, (2003).
21.El- Said F. *Nature cures in Nigeria. Part II: Lloydia*; **34(1)**, 172, (1971).
22.Odebiyi O and Sofowora A. *Planta Medica;* **36(3)**, 204, (1979) .
23. Tella A. Brit. *J. Clin. Pharmacol*; **7**, 295-297, (1976).
24.Ooshima T, Osaka Y, Sasaki H, Osawa K, Yasuda H, Matsumura M. *Arch Oral Biol*; **45**, 639–45, (2000).
25.Nostro A, Cannatelli M, Crisafi G, Musolino A, Procopio F, Alonzo V. *Lett Appl Microbiol* ;**38**, 423–7, (2004).

Assessment of genetic diversity in commercially available local varieties of Brinjal at Central Gujarat (India) by RAPD Method

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Abstract: Brinjal or eggplant (*Solanum melongena* L.) is one of the most important solanaceous vegetable crop plants after tomato and potato. Random Amplified Polymorphic DNA (RAPD) analysis was applied to four commercially available local varieties of brinjal in order to assess the degree of polymorphism. A total of 103 polymorphic amplified bands were obtained from 10 decamer RAPD primers, which discriminated all the varieties. The PIC value ranged from 0.444 to 0.797. Based on the jaccard's similarity coefficient, the similarity index was observed in the range of 0.72 to 0.86. The UPGMA dendrogram based on genetic distance segregated among four varieties of eggplant into two main clusters. Brinjal gota represent cluster I, while in case of cluster II all remained varieties were present (Long brinjal, Brinjal pr and Brinjal sunmica).By using RAPD marker, tested varieties of brinjal could provides key platform for further crop improvement and cross breeding.

Key words: Genetic diversity, RAPD, *Solanum melongena* L., UPGMA

Introduction

Eggplant or brinjal (*Solanum melongena* L.) is widely cultivated as a vegetable crop in both temperate and tropical areas. According to FAO in 2010, production of eggplant is highly concentrated, with 90% of output coming from five countries[1]. China is the top producer (58% of world output) and India is second (25%), followed by Egypt, Iran and Turkey. Brinjal is the fourth most important vegetable after potato, onion and tomato in India. More than 2000 varieties of brinjal are grown in India . Amongst different states of India, the top producer of brinjal is West Bengal (28%), followed by Orissa (21%), Bihar (12%) and Gujarat (10%), as per the data of average triennium ending (TE) 2009. Brinjal is known to have ayurvedic medicinal properties and is good for diabetic patients. It has also been recommended as an excellent remedy for— those suffering from liver complaints[2].

In present time, genetic diversity in vegetable crops has its own importance to maintain the variability in important traits and characteristic. Same time genetic diversity is usually mentioned with reference to agriculture and maintaining food security. Determination of genetic diversity of any given crop species is a suitable precursor for improvement of the crop because it generates baseline data to guide selection of parental lines and design of a breeding scheme[3]. Traditionally used morphological and biochemical markers have not been found to be discriminative enough for characterization of closely related genotypes, warranting use of more precise techniques. Variation, are unaffected by environment and molecular markers detect more stage of growth and are simply inherited. Among molecular markers random amplified

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polymorphic DNAs (RAPDs) have been extensively using in genetic research owing to their speed and simplicity[4].

Randomly amplified polymorphic DNA (RAPD) markers have been used for numerous applications in plant molecular genetics research despite having disadvantages of poor reproducibility and not generally being associated with gene regions[5].RAPD techniques are a quick and effective method for producing species specific fingerprints[4&6].

The RAPD analysis has been used extensively in phylogenetic studies of bacteria, fungi and plants. The use of RAPDs for determination of genetic relationships has been demonstrated in brinjal[7-8]. RAPDs have been extensively used for identification of Indian potato cultivars[9], tomato cultivars [10-11]. Variability in eggplant was previously studied by using RAPD technique [12-14] . There is scanty information on the use of RAPD markers for examining genetic diversity in commercially available brinjal varieties which are cultivating in local areas of central Gujarat, India. Therefore, present study was aimed at analyzing four promising eggplant varieties of central Gujarat for establishing the relationship and variability using RAPD data.

MATERIALS AND METHODS

Plants materials

Four varieties of *Solanum melongena* L. viz., brinjal gota (goa), long brinjal (lon), brinjal pr (pr), brinjal sunmica (sun) procured from Vikas Hybrids Seed Pvt Ltd., Ahmedabad. Seeds materials were properly sowing in pots, once they acclimatized; young leaves were used for detecting genetic variability within and among the tested varieties of brinjal by RAPD method.

Genomic DNA Isolation

Genomic DNA was extracted from young leaf tissue following the procedure given by Doyle[15] with some modification. DNA quality and quantity were assessed on a 0.8 % (w/v) agarose gel stained with ethidium bromide and also by using a NanoDrop® ND-1000 spectrophotometer.

RAPD analysis

A total of 10 decamer primers were used for RAPD analysis. Polymerase chain reaction were performed in 25 µl system containing 2.5 µl 10 X assay buffer (10mM Tris -Cl , pH-9.0 , 1.5 mM $Mgcl_2$, 50 mM Kcl and 0.01 % gelatin), 0.5 µl Tag DNA polymerase (Bangalore Genei pvt. Ltd.), 0.5 µl of each dNTPs (dATP , dTTP , dCTP , dGTP), 2 µl of primer & 2 µl of template DNA. 17.5 µl PCR water. DNA amplification was performed in a Thermal Cycler (Eppendorf).The PCR program was started with an initial cycle of 94°C for 4 min followed by 40 cycles of 1 min at 94°C, 1 min at 37°C and 2 min at 72°C. Finally, extension was performed at 72°C for 7 min. PCR products were examined by electrophoresis on a 2 % agarose gel containing ethidium bromide (4µl per 100 ml) at 100V for 1-2 h in 0.5x Tris-borate-EDTA buffer. The amplified DNA fragments were observed under the UV Transilluminator (Lab net India).

Data Analysis

Data matrix of RAPD profiles for fragments of similar molecular weight from each individual were scored as present (1) or absent (0). Cluster analysis was performed using Un-weighted Pair Group Method with Arithmetic Averages (UPGMA)[16] [using NTSYS-pc version 2.1 [17] software. The SIMQUAL programme was used to calculate the Jaccard's coefficient value. Den-

dogram was constructed based on UPGMA clustering of a similarity matrix generated by Jaccard's coefficient. Polymorphism Information Content (PIC) for each RAPD locus was calculated based on the number of bands/ primer, as described by Weir, using the formula PIC = 1-*Pi2*, where *Pi* is the frequency of the *i*th band in the genotype examined[18]. PIC compares the polymorphism levels across markers and is used to determine the usefulness of markers for specific studies.

Result and Discussion

Random Amplified Polymorphic DNAs (RAPDs) analyses are widely used for detecting genetic polymorphism among genotypes at molecular levels in many crop species. Keeping this in view, four varieties of brinjal were subjected to RAPD analysis using 10 randomly selected decamer primers OPA 02 to OPA 11 (Table 1). A total of 112 bands were scored for 10 RAPD primers ranging from 6-19 corresponding to an average of 11.2 bands per primer. Out of these 103 bands were polymorphic with a polymorphism of about 91.96% while 9 bands were monomorphic. PIC values ranged from 0.44 (OPA 2) to 0.79 (OPA 8) with an average PIC score of 0.63. Maximum numbers of polymorphic bands (17) were obtained with the primer OPA 7 followed by primers OPA 8 which produced 16 polymorphic bands. The average number of polymorphic bands per primer was 10.3 (Figure 1).

Jaccard's similarity coefficient matrix generated a dendrogram based on polymorphism obtained with all the selected ten primers using UPGMA clustering option of NTSYS-pc 2.02j software package[18]. Jaccard's similarity coefficient showed a wide range (0.72 to 0.86) of variability (Table 2). The scale of the dendrogram constructed from the data was between 0.73 and 0.89 with a mean value of 0.81 (Figure 2).

Table 1: Selected primers along with their sequences, amplified DNA and polymorphism generated in Solanum melongena L. varieties using 10 RAPD markers.

Sr. no.	PRIMERS	5"-3" SEQUENCE	TOTAL BANDS	POLYMORPHIC BANDS	MONOMORPHIC BANDS	PIC	%
1	OPA2	5'-TGCCGAGCTG-3'	6	6	0	0.444	100.00
2	OPA3	5'-AGTCAGCCAC-3'	11	11	0	0.661	100.00
3	OPA4	5'-AATCGGGCTG-3'	13	12	1	0.710	92.31
4	OPA5	5'- AGGGGTCTTG-3'	6	6	0	0.710	100.00
5	OPA6	5'- GGTCCCTGAC-3'	7	7	0	0.444	100.00
6	OPA7	5'-GAAACGGGTG-3'	19	17	2	0.500	89.48
7	OPA8	5'-GTGACGTAG G-3'	18	16	2	0.797	88.88
8	OPA9	5'-GGGTAACGCC-3'	9	8	1	0.592	88.88
9	OPA10	5'-GTGATCGCAG-3'	11	9	2	0.743	91.66
10	OPA11	5'-CAATCGCCGT-3'	12	11	1	0.708	91.66
		TOTAL	112	103	9		

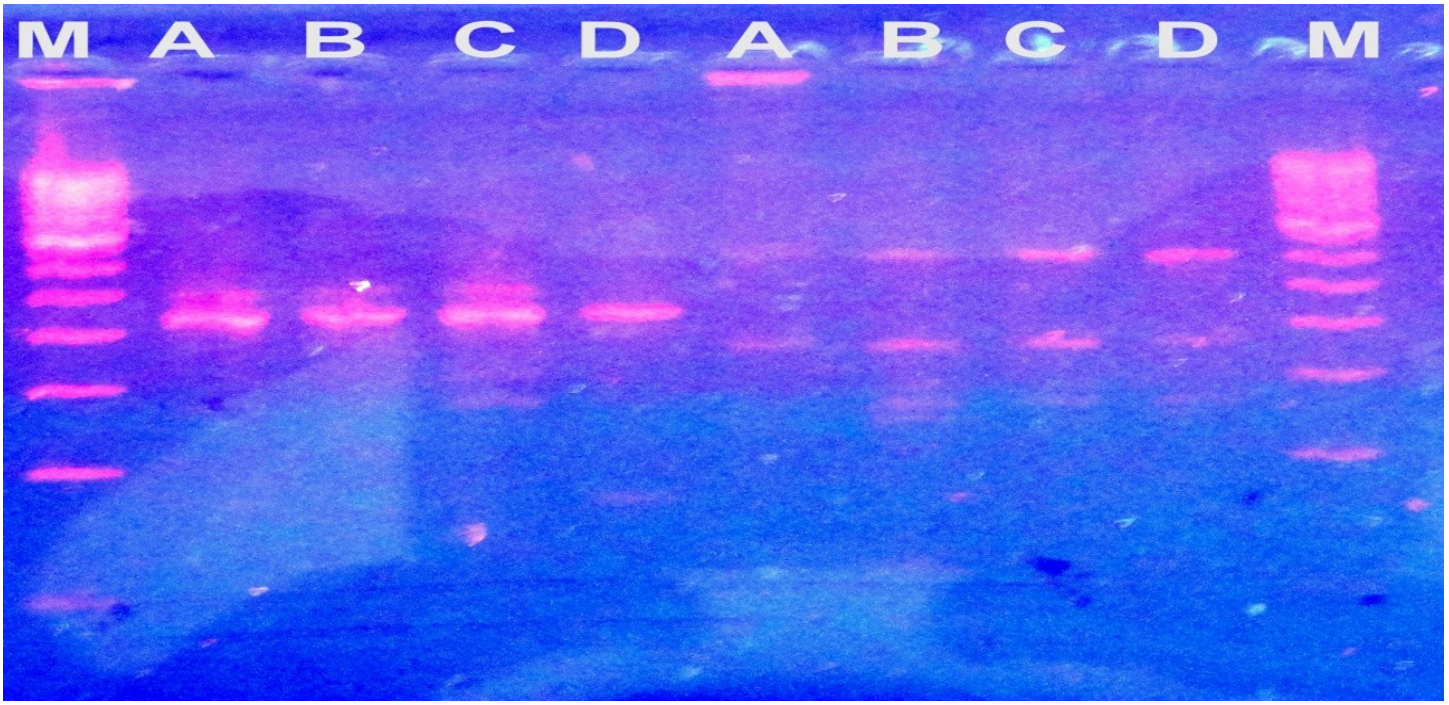

Figure 1: RAPD banding patterns of OPA-10 and OPA-11 (from left to right) in selected varieties of brinjal. The lane represents, M- Marker (100 bp to 1 kb), A-brinjal gota, B-brinjal long, C-brinjal PR and D-brinjal sumica.

The level of polymorphism observed in the present study was high going by the coefficient of variation. The correlation coefficient 0.89 for the highest similarity between genotypes and the least 0.73 exhibited a good separation from a conserved region of the genome. markers to identify genetic variability among tested varieties of brinajl. In future, these RAPD markers provide a more reliable method for identification of varieties/species than morphological characters and also provides key platform for further crop improvement and cross breeding. The UPGMA dendrogram obtained from the cluster analysis of all the tested primers showed that all four varieties of brinjal were clustered into two major clusters (Figure 2). First major cluster can further be divided into two sub cluster of which sub-cluster IIA included single variety i.e. sunmica. In case of sub cluster II B comprised of two varieties i.e. small brinjal and long brinjal. However, second major cluster included single variety that is brinjal gota. This observation is in agreement with the finding of Oyekunle[19]. Similar lines of data were also reported by previous workers on *Solanum*[20-22].

However, these are not in agreement with some earlier workers; for instance, Karihaloo[23]studied variation among the cultivated and weedy taxa of *S. melongena* by allozymes and RAPD analyses; also Ge[24] examined the genetic diversity and relationships among eggplant accessions collected from seven areas in China using SSR markers. These authors observed little or moderate amount of genetic polymorphism among the genotypes studied; even Karihaloo and Gottlieb -(1995) suggested the existence of a very small gene pool from which the cultivated forms of *S. melongena* arose.

In conclusion, the present study data reviled that the OPA4, OPA7, OPA8, OPA9, OPA10 and OPA11 RAPD primers could be used as a RAPD

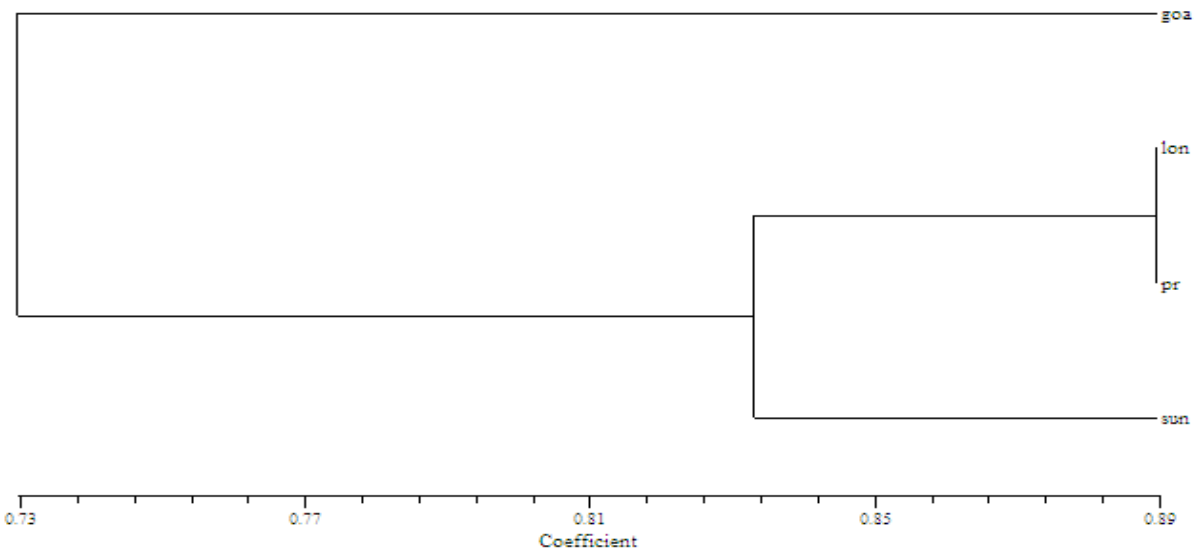

Figure 2: UPGMA- based dendogram showing genetic relationship among four brinjal varieties based on Jaccard's coefficient similarity, estimates for RAPD data.

markers to identify genetic variability among tested varieties of brinajl. In future, these RAPD markers provide a more reliable method for identification of varieties/ species than morphological characters and also provides key platform for further crop improvement and cross breeding.

Refrences

1. FAO Global forest resources assessment , Main report, Rome, Italy *FAO Forestry* **163**. (2010).
2. Shukla, V. and Naik, L.B., *Advances in Horticulture,* **5,** 365-372, (1993).
3. Vand dar Maesen LIG, *The Pigeon Pea*, **15 -46**, (1990).
4. Williams, J.G., Kubelik, A.R., Rafalski, J.A. and Tingey, S.V., *Nucleic Acids Res.,* **18,** 6531-6535, (1990).
5. Welsh, J. and McClelland, M., *Nucl. Acids Res*., **18**, 7213-7218, (1990).
6. Cipriani, G., Bella, R. and Testolin, R., *Euphytica*, **90**, 169-174, (1996).
7. Khan, M.A., Rather, T.H., Dar, A.M. and — Kudesia, R., *International Journal of Current Research*, **5(5)**, 1221-1223, (2013).
8. Tiwari, S.K., Karihaloo, J.I., Hameed, N. and Gaikwad, A.B., *J. Plant Biochemistry & Biotechnology*, **18(2)**,1-7, (2009).
9. Chakrabarti, S. K., Pattanayak, D. and Naik P.S., *Potato Research,* **44:** 375-387, (2001).
10. Noli, E., Conti, S., Maccaferri, M. and Sanguineti, M.C., *Seed Science and Technology,* **27**, 1-10,(1999).
11. Archak, S., Karihaloo, L. and Jain, A., *Current Science,* **82**, 1139-1143, (2002).
12. Demir, K., Bakir, M., Sankamis, G. and Acunalp, S., *Genetics and Molecular Research*, **9 (3)**, 1568-1576, (2010).
13. Verma, M., Rathi, S., Munshi, A., Kumar, A., Arya, L., Bhat, K. and Kumar, R., *Indian J. Hort,* **69(4)**, 517-522, (2012).
14. Ali, Z., Xu, L., Zhang, D., He, X., Bahadur, S. and Yi, J**.,** *Genetics and Molecular Research*, **10 (2)**, 1141-1155,(2011).
15. Doyle, J.J. and Doyle, J.L. *Focus*, **12**, 13-15, (1990).
16. Sneath, P.H.A. and Sokal, R.R., Numerical

Taxonomy. Freeman, San Francisco, Cali fornia, (1973).
17. Rohlf, F.J. ,NTSYS-pc numerical taxonomy and multivariate analysis system. Version 2.02e. Exeter Software, Setauket, New York, (1998).
18. Weir, B.S.,Genetic Data Analysis: Methods for discrete population genetic data. Sunderland, Massachusetts: Sinauer Associates, Inc. **377**.(1990)
19. Oyekunle, S.M., Adebayo, O.L., Olajide, A.K., Olufunmilayo, O.B. and Temitayo, O.O., *International Journal of Genetics and Molecular Biology,* **6(1)**, 1-7, (2014).
20. Furini, A. and Wunder, J., *Theor. Appl. Genet*., **108**,197-208, (2004).
21. Singh, A.K. Singh, M., Singh, A.K., Singh, R., Kumar, S. and Kalloo, G., *Current science*, **90 (5)10**, 711- 716, (2006).
22. Levin, R.A., Myers, N.R. and Bohs, L., *Amer. J. Bot.,* **93(1)**, 57-169, (2006).
23. Karihaloo, J.L., Brauner, S. and Gottlieb, L.D., *Theor. Appl. Genet.*, **90**, 767-770, (1995).
24. Ge, H., Liu, Y., Jiang, M., Zhang, J., Han, H. and Cheng, H., *Sci. Hort.,* **162**, 71-75, (2013).


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