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Biological Sciences

Comparative evaluation of Indigenous traditionally used herbal tooth sticks (Datun) and toothpaste in cleaning of mouth microflora

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ABSTRACT:

There are many plants, which are used as chewing sticks (*Datun*) in different parts of India and the world. Numerous studies have been reported on the antimicrobial effects of chewing sticks on oral bacteria. The aim of this study was to compare the antimicrobial effect of extract of seven different chewing sticks of India. The agar well diffusion Method was used to test the antimicrobial activity of seven Asian chewing sticks. It was found that at there was antimicrobial effect on pyorrhea causing bacteria at IC_{50} concentration of *Kharijal* (*Salvadoraoleodes*) from India. The inhibition zones were found in those two chewing stick extracts. It is recommended that the chewing sticks can be a great help in developing countries with financial constraints and limited oral health care facilities for their populations. Due to Indian traditional knowledge (ITK) this experiments can help to poor and make mission *make in India* more successful.

Key words: chewing sticks, antimicrobial activity**,** *in vitro,*Minimum Inhibitory Concentration,Datun, Kharijal

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There are many plants, which are used as chewing sticks (*Datun*) in different parts of India and the world. Numerous studies have been reported on the antimicrobial effects of chewing sticks on oral bacteria. The aim of this study was to compare the antimicrobial effect of extract of seven different chewing sticks of India. The agar well diffusion Method was used to test the antimicrobial activity of seven Asian chewing sticks. It was found that at there was antimicrobial effect on pyorrhea causing bacteria at IC_{50} concentration of *Kharijal* (*Salvadoraoleodes*) from India. The inhibition zones were found in those two chewing stick extracts. It is recommended that the chewing sticks can be a great help in developing countries with financial constraints and limited oral health care facilities for their populations. Due to Indian traditional knowledge (ITK) this experiments can help to poor and make mission *make in India* more successful.

Key words: chewing sticks, antimicrobial activity**,** chewing sticks, *in vitro*

Introduction:

A teeth cleaning twig or *datun* is a tool made from a twig from a tree. It can help to prevent tooth decay and gum disease.Most commonly plants are used that have a high content of tannins (astringent and antibacterial) or other compounds that benefit the health of gums and teeth.Teeth cleaning twigs can be obtained from a variety of tree species.Although many trees are used in the production of teeth cleaning twigs, some trees are better suited to clean and protect the teeth, due to the chemical composition of the plant parts. In India major five trees are most utilized as a tooth cleaner (Babool, Bunyan tree, *Karanj*, Pomegranate, Neem, *Borsali* and *Kharijal*). Oral hygiene measures have been practiced by different populations and cultures around the world since antiquity. The evolution of the modern tooth- brush has its origin in chewing sticks that were used by the Babylonians as early as 3500 BC (Wu et al.,2001).

Based on its previous herbal studies and ancestral knowledge, we designed our experiments to check its activity on mouth microflora. The aim of this study is preparation of powder from bark of tender twinges and develops MIC-Minimum Inhibitory Concentration for mouth microbes for gum diseases like Peoria.

The aim of this project is to validate indigenous traditional knowledge and ethanobotanical believes of Indian people. This study is based on Indian traditional knowledge (ITK). This era is moving towards the utilization of organic food and resources, as a proof of that herbal sticks are also available online at many different sites at INR 100 for 10 sticks. As an output we can provide scientific recommendation for tooth cleaning and better utilization of tender twinges.

Methodology:

All the experiments were carried out at food testing laboratory and Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India.

1. Collection of herbal plant materials

All plants were collected from Junagadh agricultural University, Junagadh Gujarat-362001. The tender twinges were cut and bark was removed from twinges.

2. Extract preparation from bark of herbal plants (Kang et al., 2011)

Materials:

- 10gm of Medicinal plant powder
- Methanol (AR)
- MiliQ Water
- Whatman Filter paper
- Funnel
- Beaker
- Flask

Methods:

A. Wight 10gm Medicinal plant powder
B. Prepare Methanol: Water (80:20) Solution
C. Add 120ml Methanol: Water (80:20) Solution in 10gm of medicinal plants
D. Incubate for 24hrs at 40^0C Temperature
E. Filter the mixture through Whatman filter paper
F. Air dry Filtrate and collect extract of medicinal plants
G. Store at 4^0C For Further Analysis

3. Isolation of pyorrhoea causing Pathogen

Materials:

- 1 Erlenmeyer flask containing 50ml of sterile NA broth
- 1 pyorrhoea infected teeth
- 1ml pipettes
- Wire loop
- Petri plates containing about 15ml of NA (Nutrient agar)
- paper towel
- disinfectant
- marking pens

Method:

A. Collect push from pyorrhoea infected teeth by using wire loop
B. Strick the collected bacteria on NA Plates
C. Inoculate bacteria in NA broth and Shake well
D. Incubate the Petri plates at 37^0C temperature.
E. Observe after 24hours.
F. Preserve at 4^0C For Further Analysis

3. Antibacterial activity by agar well diffusion Method

A. 15-20 mL of N- agar was poured on glass petro plates of same size and allowed to solidify.
B. Agar surface of each plate was streaked by a sterile cotton swab with the reference bacterial strain.
C. Agar plate was punched with a sterile cork borer of 4 mm size and 100 µL of each sample was poured with micropipette in the bore.
D. The plates were allowed to standby for 30 min. The plates were incubated at 37°C for 24-48 h.

Activity index was calculated by =Zone of inhibition of extract/ zone of inhibition of Antibiotic

4. Minimum Inhibitory Concentration (MIC) (Kang et al., 2011)

The Minimum Inhibitory Concentration (MIC) is determined by inoculating the organism into a series of test tubes, usually 5, that contain a standard amount of broth and serial dilutions of the antibiotic being tested. Following a period of incubation, the wells are examined for growth.

A. This assay consists in the determination of chemical agent spectrum of action, according to resistance of studied microorganisms.
B. It was developed the determination of minimum inhibitory concentration (MIC) for every Medicinal plant extracts, through the classic method of successive dilution. In Five numbered tubes, 1 ml of Nutrient Broth medium was distributed for every tube.
C. The tubes were submitted to autoclave under constant pressure and temperature of 121 °C.
D. For the first and the second tubes of the series, 1 ml of tested medicinal plant extracts was added; tube 2 was stirred and 1 ml was withdrawn and transferred for tube 3.
E. This successive transference was repeated until tube 5ml of inoculation (tested microorganism) at known concentration.
F. Incubation at optimal temperature was developed for 24 and 48 hours.
G. After this period, the reading was developed; the MIC is the concentration of the higher dilution tube in which the absence of bacterial growth occurred. Tubes 6 are controls (NA + inoculation) and Blank (NA)

H. Take optical density at 600nm.

Calculate % survival by $= \frac{\text{O.D of Treated}}{\text{O.D of Control}} \times 100$

I. Plot Graph of % Survival vs. Concentration (µg/ml). Calculate IC_{50} from Graph.

Results and Discussion:

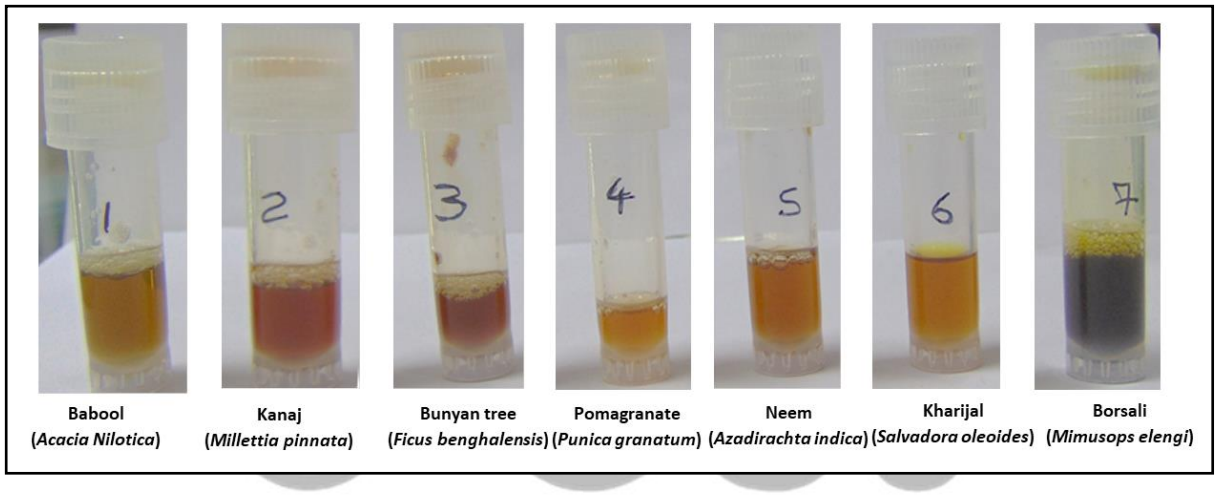
1. Collection of herbal plant materials:

All plant materials were taken from campus of Junagadh Agricultural University, Junagadh. The bark of sticks removed and collected for extract preparation Table 1.

2. Extract preparation from bark of herbal plants:

Total 7 extract were prepared from bark and collected in different vials for antimicrobial activity test Figure 1.

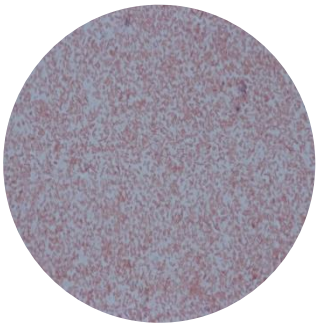
Figure 1: Extract from bark of herbal plants



3. Isolation of pyorrhea causing Pathogens:

The pyorrhea is serious gum infection. Here, we select pyorrhea for antimicrobial activity against various herbal extracts. Pyorrhea causing pathogens were isolated from infected teeth from patient. The major three isolates were found and stained as gram positive bacteria with rod and cocci shape Figure 2.

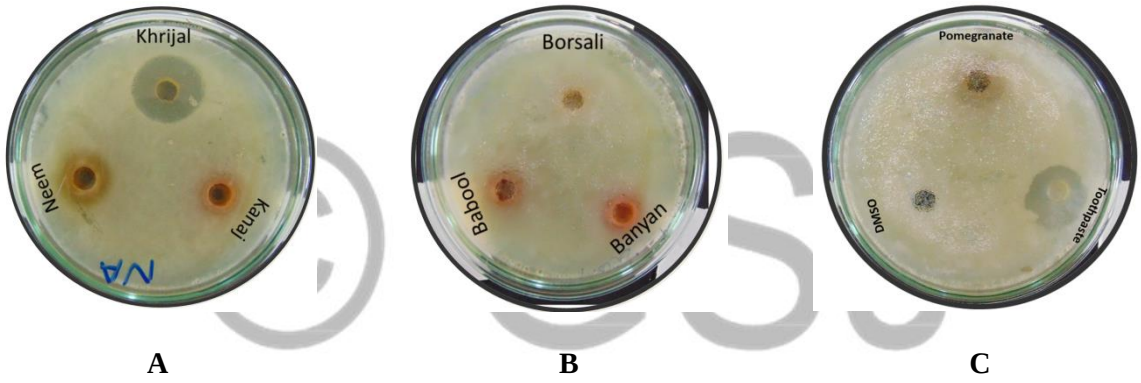
Figure2: Gram staining of Pyorrhea causing pathogens



4. Antimicrobial Activity

Seven plant species were selected for investigation to evaluate their antibacterial activity against pyorrhea causing bacteria using agar well diffusion method. Commercial toothpaste was taken for comparison. Evaluation of antibacterial activity of these plants extract and toothpaste was recorded in Table 2 and illustrated in Figure 3. The results revealed that all plant extracts were potentially effective againstpyorrhea causing bacteria with variable potency. *Kharijal* was the most effective extract retarding microbial growth of tested pathogenic bacteria, followed by toothpaste and Neem extract.Bunyan tree showed lowest zone of inhibition against pyorrhea causing bacteria.While remaining plants extract showed moderate antimicrobial activity against pyorrhea causing bacterial strain.

Figure 3: Antimicrobial activity of Herbal extract against Pyorrhea causing pathogens



A **B** **C**

4.Minimum Inhibitory Concentration (MIC)

The MIC of the most effective plant extracts were employed to evaluate their bacteriostatic properties. The concentration effect of the effective plant extracts were recorded in Table 2. The inhibitory effect of *Kharijal* extract and toothpaste started at 0.5 mg/ml, while Bunyan tree extract suppressed bacterial growth of strains at high concentration (5000 µg/ml) (Table 3).So overall *Salvadoraoleoides*plant have good results for Minimum Inhibitory Concentration.

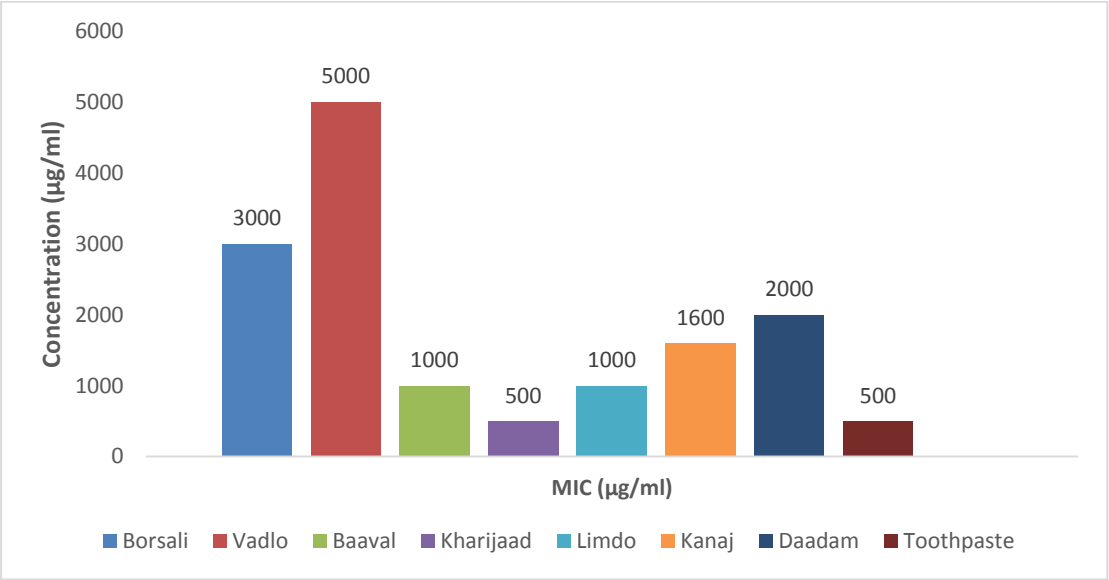
Table 2: Zone of Inhibition and activity index of 80% Methanolic extract of Different herbal extractcompare to broad spectrum of antibiotics against Pyorrhea causing pathogen

Plant (5mg/ml)	Zone of Inhibition (mm)	Activity index							
		GEN 26mm	AMP	CTR	AK 24mm	CEP	CIP 28mm	COT	CXM 12mm
Borsali	13	0.5	-	-	0.542	-	0.464	-	1.083
Vadlo	12	0.461	-	-	0.5	-	0.428	-	1.000
Baaval	14	0.538	-	-	0.583	-	0.5	-	1.167
Kharijal	25	0.961	-	-	1.041	-	0.893	-	2.083
Limdo	19	0.73	-	-	0.792	-	0.678	-	1.583
Kanaj	14	0.538	-	-	0.583	-	0.5	-	1.167
Daadam	13	0.5	-	-	0.542	-	0.464	-	1.083
Toothpaste	22	0.846	-	-	0.917	-	0.786	-	1.833
DMSO	-	-	-	-	-	-	-	-	-

Table 3: Minimum Inhibitory Concentration (MIC)

Plant	MIC (µg/ml)
Borsali	3000
Bunyan tree	5000
Babool	1000
Kharijal	500
Neem tree	1000
Kanaj	1600
Daadam	2000
Toothpaste	500
DMSO	-

Figure 4: Minimum Inhibitory Concentration of Medicinal plant extractsagainst Pyorrhea causing pathogen



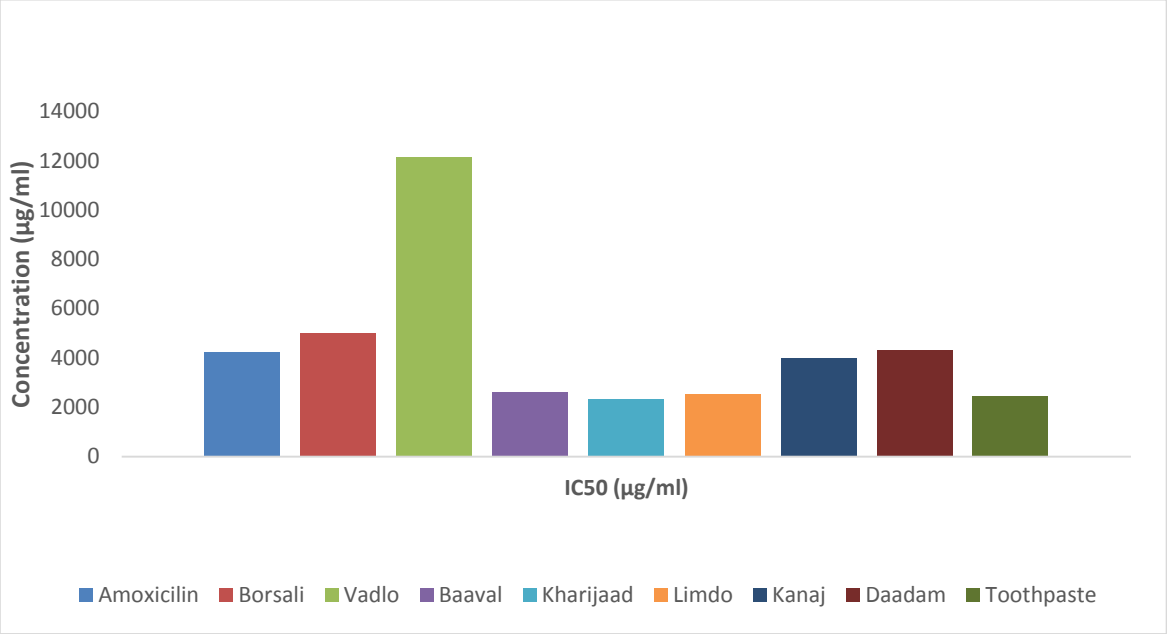
5.Comparative IC_{50} value

The IC_{50} is the concentration of an inhibitor where the response is reduced by half. The IC_{50} of extracts and toothpaste are summarized in Table 4 and Figure 5. All the seven medicinal plant extracts displayed antibacterial activity against pyorrhea causing bacteria, Kharijal displayed an lowest IC_{50} value of 2.32mg/ml , followed by the toothpaste showed an IC_{50} value of 2.45 mg/ml. Highest IC_{50} (12.17 mg/ml) was showed by Bunyan tree extract (Table 4).Among the isolated compounds indicates low antibacterial activity against pyorrhea causing pathogenic bacteria.

Table 4: IC_{50} Valueof Different herbal extractagainst Pyorrhea causing pathogen

Plant	IC_{50} (µg/ml)
Borsali	4991.875
Bunyan tree	12168.21
Babool	2632.35
Kharijal	2327.84
Neem tree	2545.714
Kanaj	3993.41
Daadam	4299.857
Toothpaste	2452.656
DMSO	-

Figure 5: Comparative IC_{50} value of Medicinal plant extracts



Discussion:

The selection of chewing sticks from India was based on a number of factors. The use of chewing sticks is most common in Asian countries especialy in the Indian subcontinent and the Middle East region, furthermore chewing sticks are cheap, readily available major area of the country. Their taste is agreeable and not unpleasant and reported to have anti-plaque and many other pharmacological properties (Lewis, 1990). So it was important to find out the antimicrobial properties of those chewing sticks as they are so commonly used in India. It is claimed that the mechanical plaque-removing properties of chewing sticks may be similar to that of a conventional toothbrush (Hardie and Ahmed 1995, Hattab 1997). Results from epidemiological study in Saudi Arabia have shown that the periodontal treatment need is low in habitual chewing stick users (AL KHATEEB et al 1991). Most studies on chewing sticks have been carried out in India, Nigeria and Pakistan where more than 90 % of the population uses different types of sticks from trees that grow there such as Babool, Bunyan tree, *Karanj*, Pomegranate, Neem, *Borsali* and *Kharijal* and others. Certain chewing sticks including those derived from *S. oleodes*, *A. indica* and *A. arabica* are active against several types of cariogenic bacteria frequently found in the human oral cavity (Akpata ES and Akinremisi EO 1997).Results from the present study showed that Kharijal (*SalvadoraOleodes*) and Neem (*A. indica* from India) had some antimicrobial activity against Pyorrhea causing pathogens at MIC concentration of the miswak extracts. This is in accord with the previous studies as far (AL BAGIEH and ALMAS 1997) as Salvadorapersica from Saudi Arabia is concerned. It may be attributed to the fact that it took almost one month to test the antimicrobial activity of chewing sticks extracts and they were not fresh, at the time of experiment. But so far none of the studies have shown the antimicrobial activity or effect of age of Asian chewing sticks. The use of the chewing stick conforms with the notion of primary health care approach (PHCA) and the well-established associations with certain cultural and religious beliefs.

The chewing sticks have been proven effective as an oral hygiene tool and its use should be promoted with scientific rational and proper method of its preparation and usage. The use of chewing sticks will be a great help in developing countries with financial constraints and limited oral health care facilities for their populations.Several researchers investigated the efficiency of plant extracts and their effective compounds as antimicrobial agents to control growth of Pathogenic bacteria. Some researchers have suggested that antimicrobial components of the plant extracts (terpenoid, alkaloid and phenolic compounds) interact with enzymes and proteins of the microbial cell membrane causing its disruption to disperse a flux of protons towards cell exterior which induces cell death or may inhibit enzymes necessary for aminoacids biosynthesis. Other researchers attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plants extracts which enable them to react with protein of microbial cell membrane and mitochondria disturbing their structures and changing their permeability (Friedman et al., 2004; Tiwari et al., 2009). The present

study suggested that plant extracts which proved to be potentially effective can be used as natural preservatives to control mouth diseases and avoiding application of healthy hazards of chemical paste etc.

Conclusion:

On the basis of previous evidences, It is concluded here that Kharijal (*Salvadora oleoides*) is the best among herbal teeth cleanings sticks followed by toothpaste , Neem tree, and Babool in that order.

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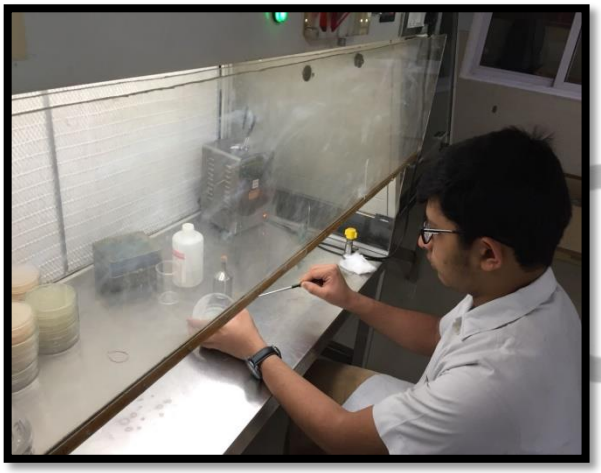
Table 1: Herbs utilized for experiments

Name of Herbs	Babool (*Acacia Nilotica*)	Kanaj *(Millettia pinnata)*	Bunyan tree *(Ficus benghalensis)*	Pomagranate *(Punica granatum)*	Neem *(Azadirachta indica)*	Kharijal *(Salvadoraoleoides)*	Borsali *(Mimusopselengi)*
Photos							
Tooth sticks							
Bark							
Powder							

SOME HIGHLIGHTS DURING LAB WORK



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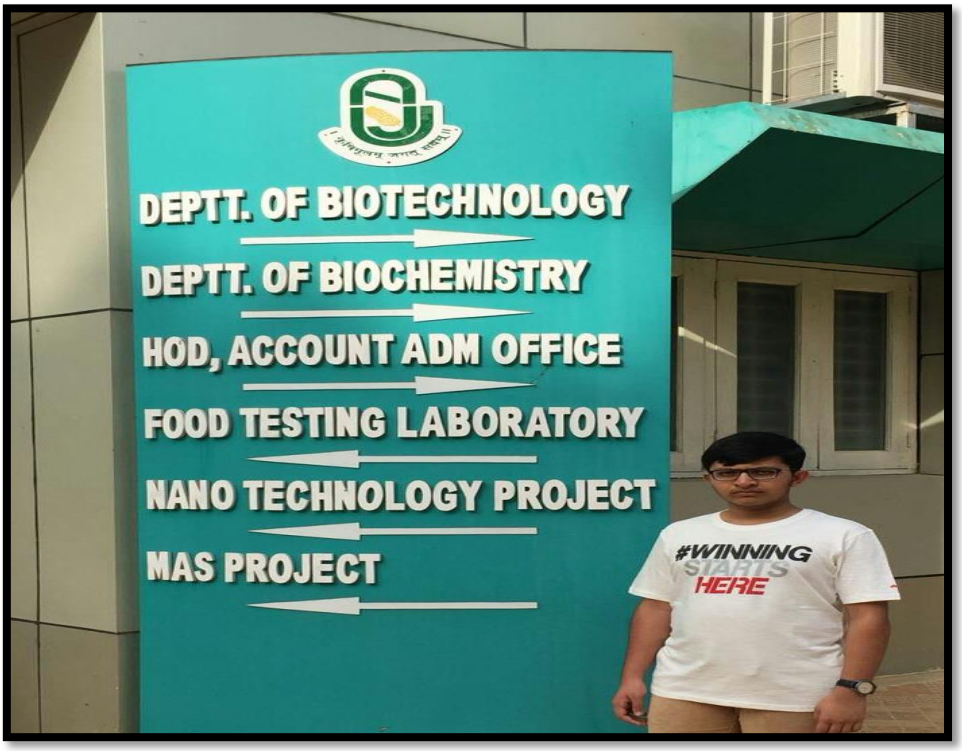

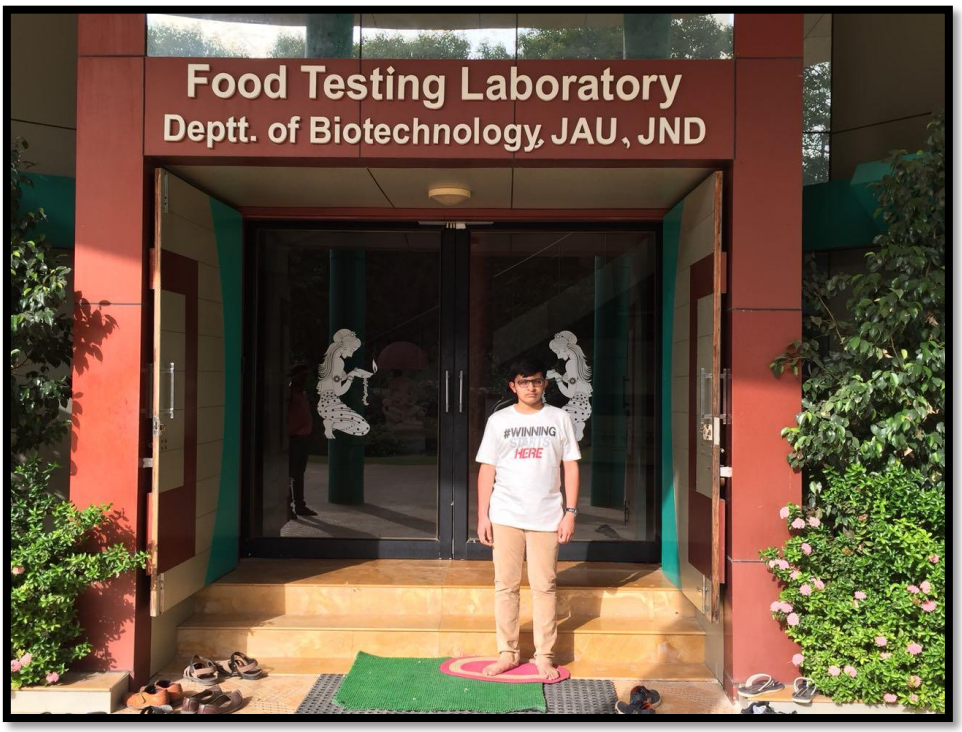


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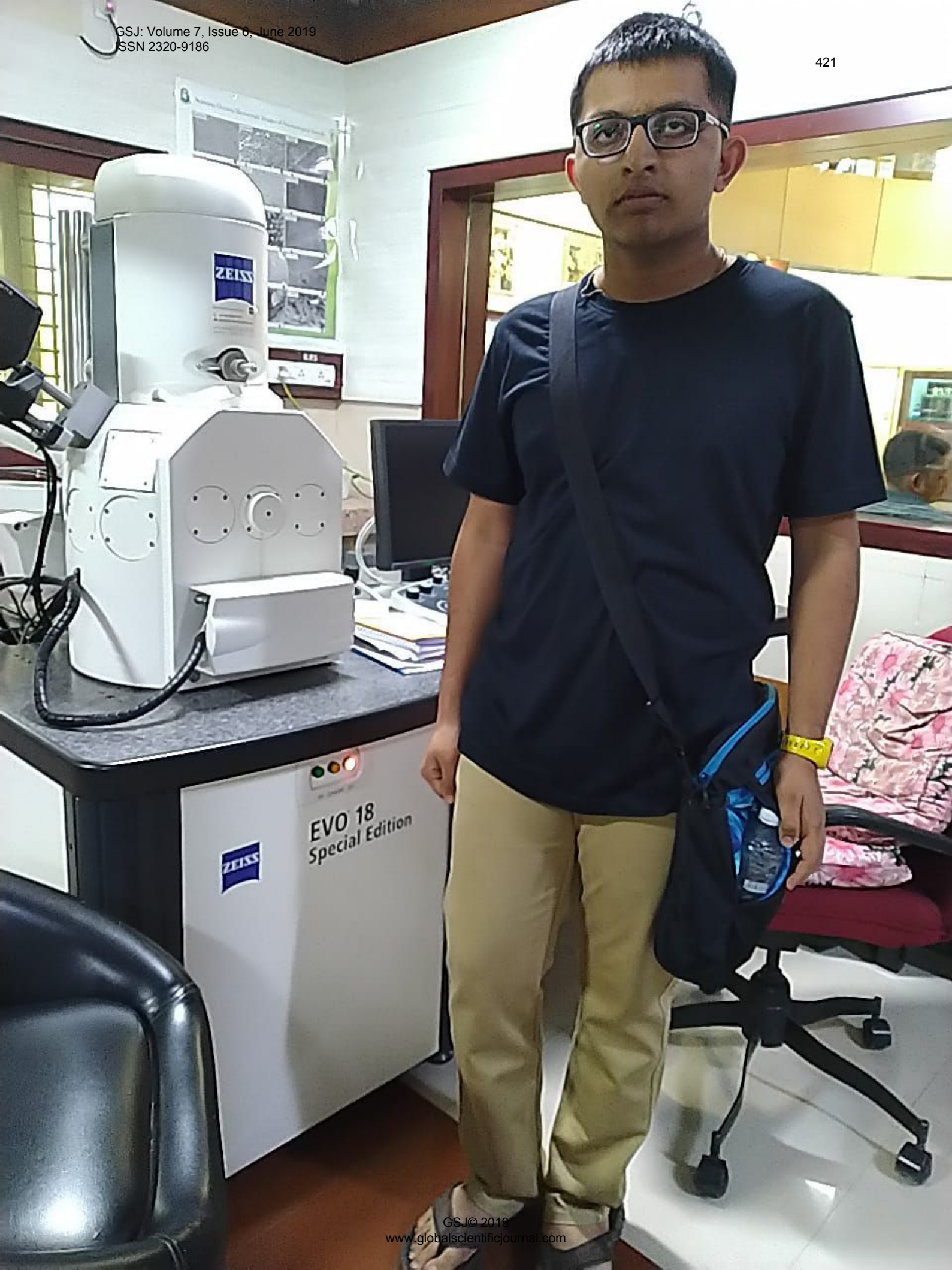
HiCare


Vapodest


HiCare


HiCare


FLAGYL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Hello,

Congratulations! You have been shortlisted for the next round of IRIS National Science Fair.

We would like to hear more about your project and what you have learnt from it, through a Skype call.
For this, you will need to create a Skype ID and share the ID with us.

The calls will be scheduled for 5th and 6th of October, 2018. Since this round of selection is essential to move forward, we would like for you to attend the call.
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Kindly reply to this email with your confirmation as well as your Skype ID, as soon as possible, following which we will share the date and time scheduled for the call.

If you have any queries or concerns, please reach out to the following numbers:
- Shray: 9717194824
- Sincy: 9446691720
- Shinoy: 8951994249

Thank you and good luck!

Best Wishes,

Team IRIS

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॥ यथा पिंडे तथा ब्रह्मांडे ॥

CERTIFICATE

This is to certify that Mr./~~Ms.~~ **Dhyey Dharmendrakumar Mavani** student of P. P. Savani International School, Surat has successfully completed the training on **"Testing of antimicrobial activity of herbs"** during 15/05/2018 to 14/06/2018 at Food Testing Laboratory of Department of Biotechnology, J.A.U., Junagdh.

The student is found sincere, keen learners and disciplined in their overall conduct. I wish him/~~her~~ all the best for future endeavors.

Date : 15/06/2018
Place : Junagadh

॥ अन्नम् ब्रह्म ॥

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