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A comparative pharmacognostic and antimicrobial evaluation of different parts of *Mimusops elengi* for dental associated problems

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Abstract
Herbal drug attaining wide acceptance day by day because they are safe, very effective and less costly. The utilization of plant as a medicine is mentioned in Ayurveda, Siddha, Unani medicines etc but the therapeutic properties of most of them have not be proven scientifically. Therefore, Pharmacognostic and Phytochemical evaluation of *Mimusops elengi* L, is carried out to know the complete information of crude drug. *Mimusops elengi* L. (*Sapotaceae*) is an important medicinal plant because each and every part is used in treatment of various ailments. In this study ethanol extract of *Mimusops elengi* (*M. elengi*) plant parts like bark, leaves and seeds were examined for antimicrobial activities against micro-organisms (*Streptococcus mutans, Streptococcus aureus,* Yeast (*Saccharomyces cerevisiae)* frequently involved in dental associated problems as well as Plaque (fresh as well as stored). The antibacterial potential of both the plants was confirmed of this study indicated that, the ethanol extract of *M. elengi* bark and leaves was found to be very effective and seeds gave lesser activity as compare to bark and leaves. Also very effective activity against *Saccharomyces cerevisiae* (*Yeast*). Since the antimicrobial activity of ethanolic extract of bark, leaves and seeds *M. elengi* was evaluated by the standard Well diffusion and broth dilution method.

Keywords: *Mimusops elengi*, *Saccharomyces cerevisiae*, *Streptococcus mutans*, *Streptococcus aureus*, Dimethyl sulfoxide

Introduction
Plants were considered as factories which biosynthesize lot of chemical compounds such as alkaloids, glycosides, saponins, resins, lactones and oils which act on human body. Herbal medicines are employed to cure and prevent a wide variety of health related problems ranging from treatment of common colds to treatment of Cancer [1]. The practice of traditional medicine is based on hundreds of years of belief and observations and analysis which help in the development of modern medicine. Today whole world is showing interest in herbal medicines [2]. The use of plants and plant products in medicines is getting popularized because the herbal medicines are easily available and have natural origin with higher safety margins and lesser or no side effects [1]. *M. elengi* contains active components showing various kinds of biological and pharmacological activities. It had some biological and pharmacological activity such as antibacterial, antifungal, anticarcinogenic, antihyperglycemic,antiviral antihemorrhoidal and cytotoxic activites. It had been previously reported as antiulcer, antiinflammatory, antianxiety, antihyperlipidemic, anticonvulsant, analgesic, antipyretic, antioxidant, cytotoxic. Chewing of a twig of *M. elengi* is as Danta Pavana and is useful for cleaning teeth. Unripe fruit and seed was used to fix loose teeth [3]. The bark was used as a tonic, and in gargles to cure odontopathy, inflammation and bleeding of gums [1].

Oral hygiene is an integral part of general health of a person. Oral disease can significantly affect the general well-being of a person by causing considerable pain and discomfort, thus affecting quality of life [4]. Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. The association between oral diseases and the oral micro biota is well established. The development of dental caries involves acidogenic and aciduric Gram-positive bacteria (*Mutans Streptococci, Lactobacilli and Actinomycetes*). Periodontal diseases have been linked to anaerobic Gram-negative bacteria (*Porphyromonas gingivalis, Actinobacillus, Prevotella and Fusobacterium* [5]. Due to the growing evidence of relation between oral health and whole body health dental practitioners may seek to respond to their patient's oral hygiene needs with newer products. These research based products come with naturally occurring active ingredients, that achieve the desired antibacterial and anti-inflammatory effect [6]. The plant extract or phytochemicals that hinder the growth of oral pathogens, diminish the progress of dental plaque, manipulate

The adhesions of bacteria to surface and reduce the symptoms of the oral diseases [7].

Materials and Methods

Collection and Authentication of Plant Material

The plant material was procured from local herbal market of Nagpur, Maharashtra. The plant specimen were dried and their herbarium sheet was prepared. It was botanically authenticated by Dr. Nitin Dongarwar, Professor, Department of Botany, R.T.M. Nagpur University, Nagpur. The specimen voucher number of the plant was 1030.

Preparation of Plant extract

The dried powder of bark, Leaves and seeds were extracted with Ethanol by using maceration process.

Pharmacognostic Studies

Physiochemical evaluation

The various physiochemical parameters such as total ash, acid insoluble ash, water soluble ash, extractive value and moisture content was determined as prescribed by Indian Pharmacopeia and WHO guideline[10].

Preliminary Phytochemical Screening

Phytochemical screening of Bakul bark, seeds, leaves and extracts were done for the presence of phytoconstituents such as proteins, flavonoids, saponins, steroids, glycosides, alkaloids, tannins, phenols and carbohydrates by the use of various reagents [11].

Quantitative estimation of Phytoconstituents

Based upon the Result obtain from Preliminary Phytochemical Screening. Quantitative estimation of detected phytoconstituents like total Phenolic content [14], total tannin content [15], total alkaloid content [16], total Saponin content [17]. Total carbohydrates content [18], total steroid [19], total Flavonoid content [20] were carried out by standard procedure.

Fingerprinting analysis of Bakul (seeds, leaves and bark)

Thin layer chromatography [16]

The Arks were subjected to thin layer chromatographic studies, to find out the probable number of compounds present in them. The details of the procedure are as follows:

Preparation of the plates

The adsorbent/stationary phase used for thin layer chromatography was silica gel G. About 25 g of silica gel G was taken in a glass mortar and about sufficient water was added to it. The mixture was stirred with glass rod until it became homogeneous and allowed to swell for 15 minutes. Then additional water was added to it with stirring. This suspension was then uniformly spread immediately on plates.

Drying and storage of plates

The freshly coated plates was then air dried and stacked in a drying rack and was heated in an oven for 30 minutes at 110 °C. Activated plates was kept in a desiccator, till required for further use.

Sample and Standard preparations

Sample and Standard stock solution where prepared in Methanol.

Application of the sample

The test samples were applied in the form of a band, with the help of fine capillaries.

Developing solvent system

Table 1: developing solvent system

Plant Parts	Solvents Systems.
Bark	Toluene: Ethyl acetate: Formic acid (8:2:0.1) Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2) Chloroform : Methanol: Formic acid (8:1:1)
Leaves	Toluene: Ethyl acetate: Formic acid (8:2:0.1) Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2) Chloroform : Methanol: Formic acid (8:1:1)
Seeds	Toluene: Ethyl acetate: Formic acid (8:2:0.1) Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2) Chloroform : Methanol: Formic acid (8:1:1)

Development of TLC plates

Chromatographic rectangular glass chamber was used in the experiments to avoid insufficient chamber saturation. Different mobile phase where tried but the satisfactory resolution was obtained in the solvent systems mentioned in figure 2 and 3. After development of plates, there was air-dried and numbers of bands was noted & R_f (Retention Factor) values was calculated.

The R_f value was calculated as follows.

$$R_f = \frac{\text{Distance traveled by the sample}}{\text{Distance traveled by the solvent}}$$

Conformation of B-Sitosterol and Ursolic Acid by Thin Layer Chromatography

Thin layer chromatography was performed for *Mimusops elengi* Bark, Leaf and Seed compared with standard β-Sitosterol (procured from sigma Aldrich) and Ursolic acid (procured from local herbal market) by using different solvent system hexane : ethyl acetate(7.5:2.5) and toluene : ethyl acetate : glacial acetic acid (9:1:0.4).

High Performance Liquid Chromatography (HPLC) (UFLC Shimadzu, SPD-M20A with PDA Detector) [12, 13]

Optimization of chromatographic conditions

The chromatographic studies were performed on C_{18} analytical column (Spinchotech Pvt. Ltd. Enable). Initially, different Mobile phases were tried in isocratic mode to get adequate retention of biomarkers. Different mobile phases containing Acetonitrile: Water; water: Acetonitrile was tried and finalized which gave the best resolution at less retention time. The flow rate of 1.0 ml/min was utilized and different the analysis absorption maxima for the analysis were selected on the basis of spectral study.

Standard solution

Accurately weighed quantity of (1.0mg) biomarker (β-sitosterol and ursolic acid) were dissolved in 10 ml of

methanol (100ppm) and different concentrations were prepared ,filtered with syringe filter (0.22) and utilized as working standards.

Sample preparation

Accurately weighed quantity of (1.0 mg) of extracts (*Mimusops elengi* leaf, bark and seed) were 1 ml of methanol (1000 ppm) and different concentration were prepared, filtered with syringe filter (0.22) and utilized for analysis.

Procedure:

The chromatographic conditions were set as per the given parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Working standard solution was injected in the **(**Rheodyne injection) and the chromatograms were recorded for the standard and samples

Table 2: Chromatographic conditions for HPLC in β-sitosterol

	Standard	β-sitosterol
2	Column	C_{18} G5µm 250 X 4.6 mm
3	Mobile phase	ACN: Water (90:10)
4	Detection Wavelength	212 nm
5	Injection volume	20 µl
6	Flow rate	1.0 ml/min
7	Temperature	25±2 ^{0}C

Table 3: Chromatographic conditions for HPLC in Ursolic acid

	Standard	Ursolic acid
2	Column	C_{18} G5µm 250 X 4.6 mm
3	Mobile phase	ACN:Water (89:11)
4	Detection Wavelength	240 nm
5	Injection volume	20 µl
6	Flow rate	1.0 ml/min
7	Temperature	25±2 ^{0}C

Antimicrobial studies

Collection of Important Cariogenic Organism

The strain *Streptococcus mutans* MTCC 890 was sub-cultured periodically in Mueller- Hinton Broth and was procured from Microbial Type Culture Collection, IMTECH, Chandigarh.

Plaque collection And Storage

Dental plaque samples were collected from the adult patients in Government Dental College and Hospital-Nagpur. Dental plaque sample was collected on teeth surface with help of Dental scaler and add Eppendorf tube containing phosphate buffer saline solution.

Preparation of Extract of Test Compound.

Accurately weighed quantity of (100 mg) of extracts (*Mimusops elengi* leaves, bark and seeds) with 1 ml of water and different concentration were prepared.

Evaluation of Antimicrobial Activity by Zone of Inhibition by Agar well diffusion method

Is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the Mitis Salivarius agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 mL) of the antimicrobial agent (Chlorhexidine 0.2%) or extract solution at desired concentration is introduced into the well. Then agar plates are incubated 37 °C for 24-48hrs and observed for zone of inhibition around the well [8].

Fig 1: Anaerobic Jar with Gas Pack

Determination of minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) of selected plant extract against cariogenic organisms with some modifications was determined. The assay was initiated by pouring sterile Mueller Hinton Broth (2ml) into the test tube. Exactly 2ml of 100mg/ml selected plant extract prepared in dimethyl sulphoxide (DMSO) was taken by pipette and added into first test tube containing 2ml Mueller Hinton broth and mixed using a pipette. From the first test tube 2ml of broth and plant extract was transferred to the second test tube by two-fold dilution method. Similarly 2ml broth and extract were transferred from second test tube up to sixth test. The last test tube (7th test tube) was considered as positive control (containing 2 ml Chlorhexidine instead of plant extract). Concentrated suspensions of microorganisms (0.2ml) were added in each well. Test tubes opening were sealed with aluminum foil before incubating at 37^{o}C in an incubator for 18hrs. After incubation, the development of turbidity was indicative of growth (positive indicator of cell viability). MIC values were regarded as the lowest concentrations of the extracts that inhibit the growth of the test organisms (decrease in the intensity of the clear solution). The experiments was performed in triplicate [9].

Results

Physicochemical parameters Ash value

Table 4: Ash Value

ASH VALUE (%w/w)	Bark	Seeds	Leaves
Total Ash	12.5±0.1	2±0.2	6.5±0.8
Water Soluble Ash	1.5±0.2	0.5±0.5	1±0.4
Acid Insoluble Ash	7.5±06	1±0.6	4.5±0.7

Extractive value

Table 5: Extractive value

Extractive Value (%w/w)	Bark	Seeds	Leaves
Water	21.6±0.1	18.4±0.5	17.6±08
Methanol	26.8±0.5	16.8±0.2	20±0.5
Acetone	4±0.2	10.4±0.6	4±0.7
Ethyl acetate	1.28±0.8	8.8±0.5	2.4±0.4
Petroleum Ether	0.8±0.11	10.4±0.2	16.8±0.5
Chloroform	0.48±05	16.8±0.7	6.4±0.4

Loss on Drying

Table 6: Loss on Drying

Loss on drying (Moisture content) %w/w	
Leaves	0.57±0.1
Bark	0.97±0.5
Seeds	0.77±0.3

Phytochemical Screening

Table 7: Phytochemical Screening

Plant constituent	Test Reagent	Bark	Leaves	Seed
Proteins	1) Biuret test	+	+	+
	2) Xanthoprotein test	+	+	+
Amino acid	1) Ninhydrin test	+	+	+
Carbohydrates	1) Molisch test	+	+	+
Steroid	1) Salkowski test	+	+	+
Saponins	1) Foam test	+	+	+
Alkaloids	1) Dragendorff's reagent	-	-	-
	2) Mayer's Reagent	-	-	-
	3) Wagner's Reagent	-	-	-
	4) Hager's Reagent	-	-	-
Tannins	1) Ferric chloride test	+	+	+
	2) Lead acetate test	+	+	+
	3) Potassium dichromate	+	+	+
Flavonoids	1) Shinoda test	+	+	+
	2) Sulphuric Acid test	+	+	+
Glycosides	1) Borntrager's test	+	+	+

Table 8: Quantitative Estimation

	Bark	Seed	Leave
Total phenol eq. Gallic acid	121.5 ±0.2	64.27 ±0.1	85.5 ±0.14
Total tannin eq. Tannic acid	112.5±0.5	78.49±0.11	54.27±0.8
Total saponin eq. Diosgenin	135.93±0.4	174.98±0.2	84.16±0.5
Total carbohydrate eq. Dextrose	87.15±0.8	89.44±0.7	78.99±0.2
Total steroids eq. Cholesterol	129.21±0.9	64.99±0.5	32.57±0.6
Total flavonoideq. Quercetin	67.64±0.7	8.4±0.8	3.7±0.4
Total alkoloid %(w/w)	-	-	-

Thin Layer Chromatography

Conformation of B-Sitosterol and Ursolic Acid by Thin Layer Chromatograph

Fig 2: (A) Standard Beta-Sitosterol, (B) Leaves extract, (C) Bark extract, (D) Seeds extract

Table 9: Number of Spots and Retention factor of different extract and Standard

Sample	No. of spot	Rf value
Standard	1	0.49
Bark	3	0.49,0.79,0.94
Leaves	5	0.27,0.37,0.49,0.67,0.77
Seeds	3	0.49,0.61,0.7

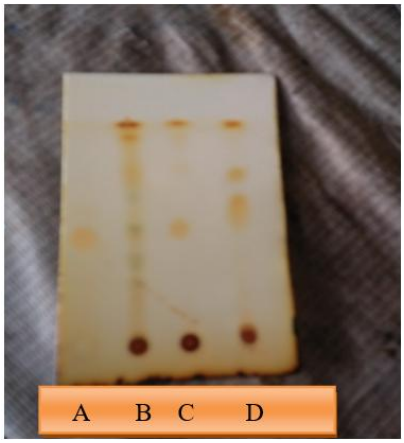

Fig 3: (A) Leaves extract (B) Bark extract, (C) Seeds extract, (D) Standard Ursolic Acid

Table 10: Number of Spots and Retention factor of different extract and Standard

Sample	No. Of spot	Rf value
Standard	5	0.1,0.3,0.35,0.53,0.79
Bark	5	1.9,0.53,0.61,0.75,0.98
Leaves	10	0.1,0.2,0.3,0.35,0.40,0.46,0.53,0.58,0.67,0.74
Seeds	7	0.14,0.24,0.4,0.53,0.64,0.77,0.80

High Performance Liquid Chromatography (Hplc)

Beta-Sitosterol

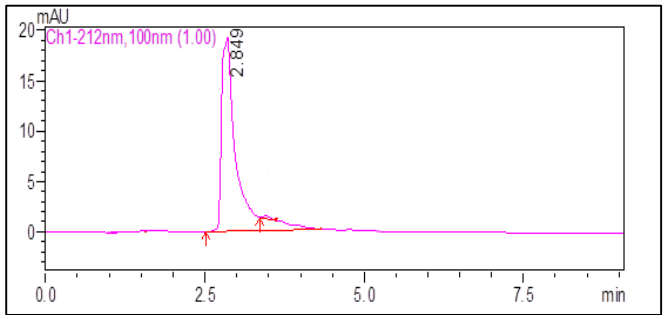

Fig 4: HPLC Chromatrogram for standard Beta-sitosterol

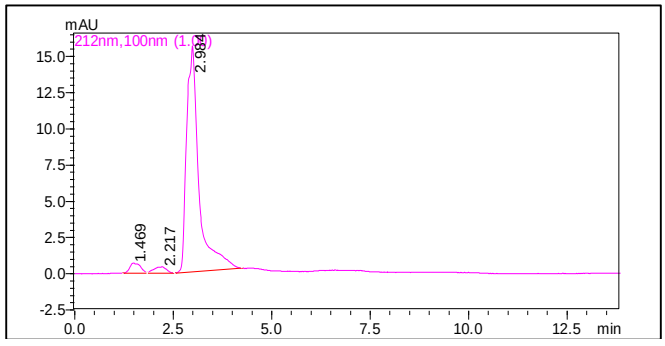

Fig 5: HPLC Chormatogram for *M.elengi* Leaves

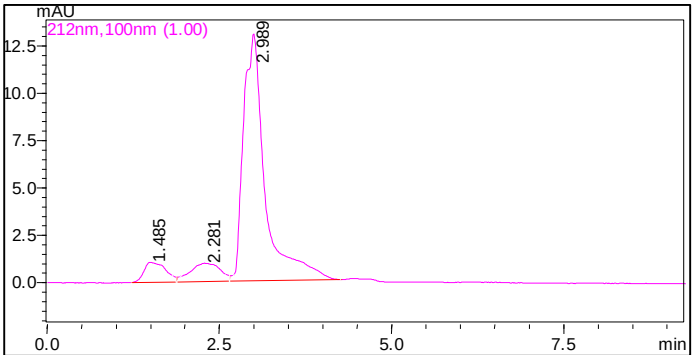

Fig 6: HPLC Chormatogram for *M. elengi* Bark.

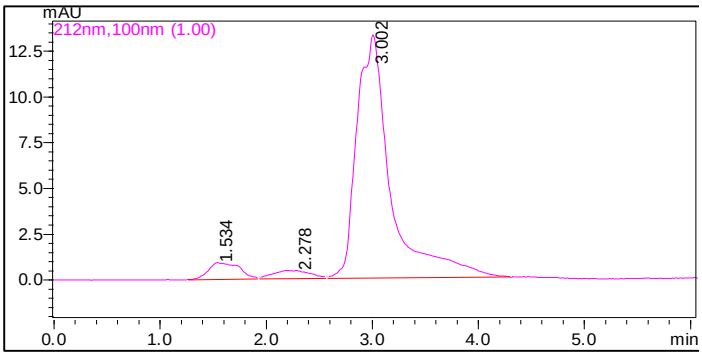

Fig 7: HPLC Chormatogram for *M. elengi* seeds.

Table 11: Retention time,Area,Conc., % of β-sitosterol present in Bark, Leaves and Seeds

Sr.no	Sample	Conc. (ug/ml)	Retention Time	Area	% of B-sitosterol
1	B-sitosterol	50	2.849	317105	-
2	Leaves	50	2.984	157159	9.53
3	Bark	100	2.989	131108	8.26
4	Seeds	100	3.002	112500	7.09

Ursolic Acid

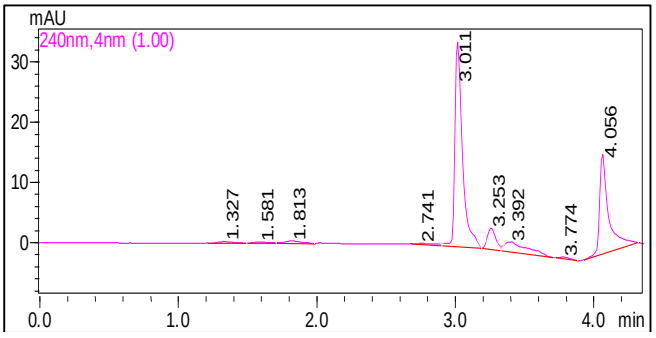

Fig 8: HPLC Chormatogram for standrad Ursolic acid

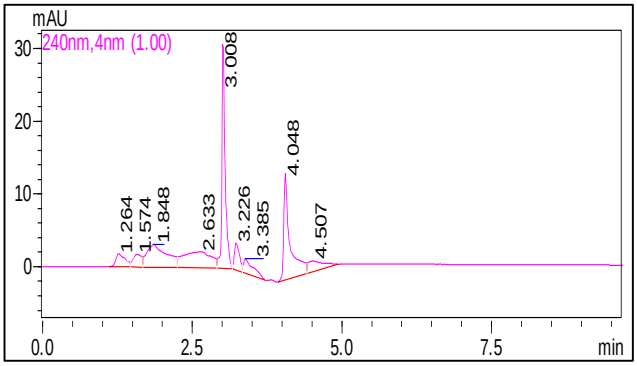

Fig 9: HPLC Chormatogram for *M. elengi* bark

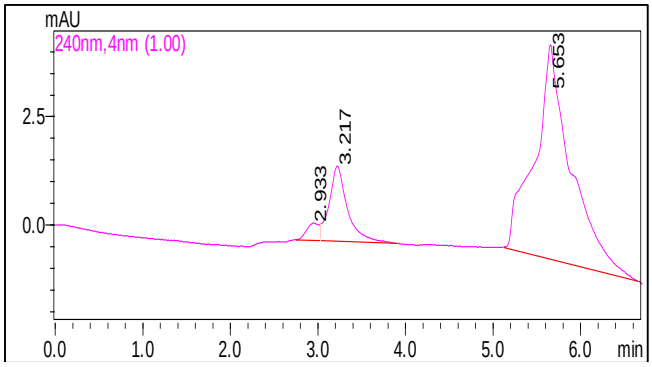

Fig 10: HPLC Chormatogram for *M. elengi* seeds

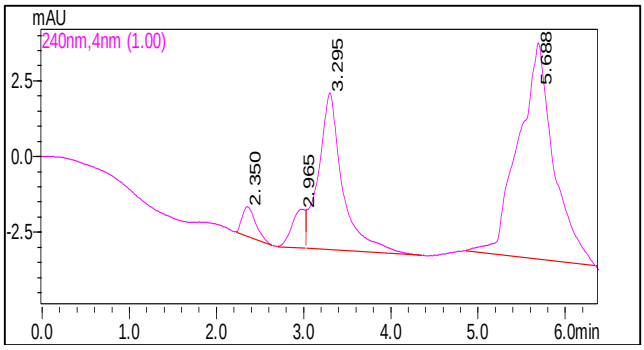

Fig 11: HPLC Chormatogram for *M.elengi* leaves

Table 12: Retention time, Area,Conc., % of Ursolic acid present in Bark, Leaves and Seeds

Sr. no	Sample	Conc. (ug/ml)	Retention time	Area	% ursolic acid
1	Ursolic acid	10	3.011	126084	-
2	Bark	100	3.008	101153	16.04
3	Leaves	100	3.295	99105	7.8
4	Seeds	100	3.217	25238	4

Table 13: Zone of inhibition against *S. mutans*, *S. aureus*, Plaques, Yeast

Bacteria	Zone of inhibition(mm)			
	Plant Extracts(100mg/ml)			Standard
	Bark (A)	Leave (B)	Seed (C)	Chlorhexidine
S. mutans	14	12.5	9	14.5
S. aureses	12.5	8.5	7	13
Plaques (fresh or Isolated)	10	7.5	6.5	11.5
Yeast (Saccharomyces cerevisiae)	11	7.5	6.5	11.5

Concentration of the each extract used: 100mg/ml, Chlorhexidine: 2mg/1ml

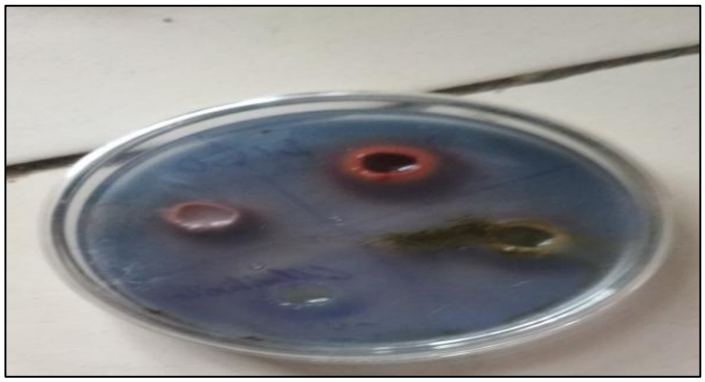

Fig 12: Zone of inhibition of Bark, Leaves, Seeds and chlorhexidine

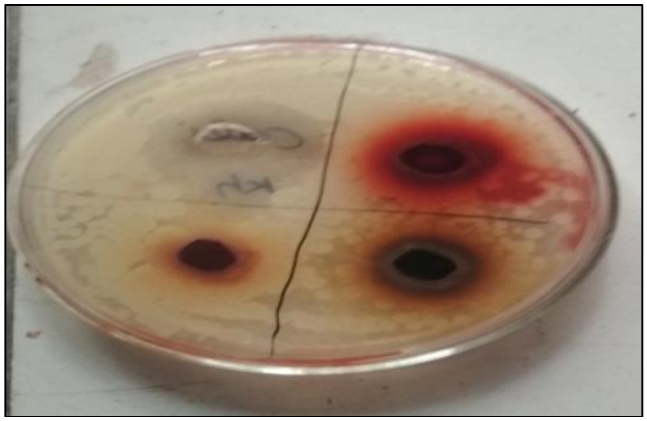

Fig 13: Zone of inhibition of Bark, leaves, seeds and chlorhexidine against

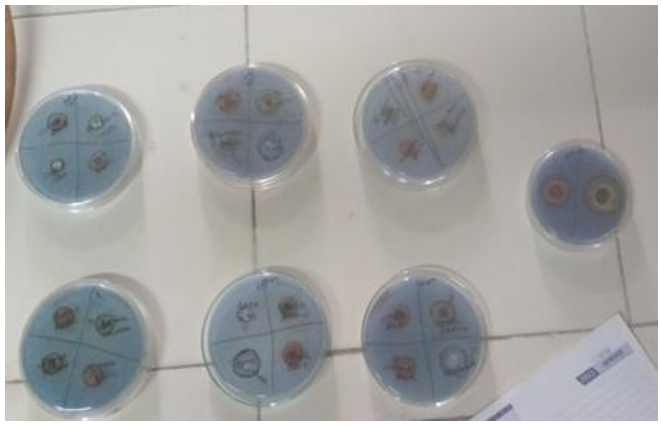

Fig 14: Zone of inhibition of Bark, Leave and Seed extract against Plaques

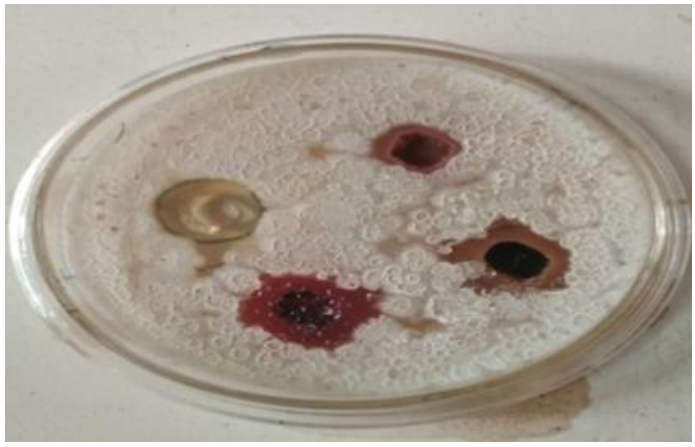

Fig 15: Zone of inhibition of Bark, Leave and Seed extract against *Yeast*

Minimum inhibitory concentration of extracts

Table 14: Minimum inhibitory concentration of extracts of *Mimuspos elengi (M. elengi).*

Sr. no.	Bacterias	Minimum inhibitory concentration(mg/ml)				
		Bark	Leave	Seed	Chlorhexidine	Blank
1	*S. mutans*	3.9	7.0	13.2	No growth	Growth
2	Plaques (fresh or Isolated)	2.7	5.8	9.8	No growth	Growth

Discussion

The present work deals with investigation of comparative study for leaves bark and seeds of *Mimusops elengi* on dental associated problems in support of traditional use.

Evaluation of pharmacognostic character, macroscopy and microscopy along with the quantitative analytical microscopy is one of the simplest and cheapest methods to start with for establishing the correct identity of the source material. Quite a significant amount of research has already been carried out during the past few decades in exploring the phytochemistry and pharmacognostic study of different parts of *M. elengi* [21].

In present study maceration extraction process was used to extract secondary metabolite of leaves, bark and seeds.

Observation from phytochemical screening showed the presence of protein, amino acid, carbohydrate, tannin, saponin, phenol, steroid, flavonoid, terpenoid, in bark, leaves and seeds. All parts of *M. elengi* were subjected to quantitative estimation such as total phenol, total saponin content, tannin content, flavonoid content, steroid content and carbohydrate content.

Help of TLC one can easily determine purity of sample, examination of reaction identification of compounds in a mixture and separation of multicomponent.

A simple, precise and accurate high-performance liquid chromatographic method. Different mobile phase are tried HPLC separation of ursolic acid and β-sitosterol from bark, leaves and seeds. Good separation was achieved by using mobile phase were water: acetonitrile and acetonitrile: water. Detection wavelength were carried out 240nm and 212nm**.** Maximum response at this wavelength. The HPLC quantification of Ursolic acid and β-sitosterol were done and it's showed in bark, leaves and seeds.

This study is attempt to strengthen studies to show the therapeutic effect on caries causing bacteria since the extract of *M. elengi* was used as an anticariogenic against two dental caries causing bacteria *S. mutans, and S. aureus. S. mutanes* were present in 95% of total cariogenic microbs. *M. elengi* extracts had antibacterial activity against the dental caries causing bacteria *Streptococcus mutans* isolated from the patients *M. elengi* bark have been tested for their antibacterial activity against dental infection causing bacteria [22]. So some literature study on bark and claimed bark is very potent. Our study find out leaves and seed also give activity against cariogenic bacteria. The present investigation showed that while MSB and muller-hinton allowed the growth of a

laboratory strain of *S. mutan s*but MSB are recovered of *S. mutanes* greater than muller hinton agar.

Fresh plaque dissolved in phosphate saline buffer gives good result as compare muller hinton broth, nutrient broth and normal saline solution. Ethanolic extract dissolved in dimethyl sulfoxide gives little more zone of inhibition as compare ethanolic extract dissolved in water.

S. mutanes are required anaerobic condition because there are not well developed in aerobic condition.

Conclusion

1. The present research attempt for the standardization and investigation of comparative study leaves, barks and seeds on *Mimusops elengi* for dental associated problems.
2. As the pharmacologists are looking forward to develop new drugs from natural sources, development of modern drugs from *M. elengi* Linn can be emphasized for the control of various diseases.
3. *M. elengi* has been reported in the literature as a good antibacterial agent and anti-inflammatory. Plants contain phytochemicals such as alkaloids, tannins, essential oils and flavanoids which have pronounced antimicrobial activity.
4. The present research attempt for the standardization and investigation of comparative study leaves, barks and seeds on *Mimusops elengi* for dental associated problems.
5. As the pharmacologists are looking forward to develop new drugs from natural sources, development of modern drugs from *M. elengi* Linn can be emphasized for the control of various diseases.
6. Ursolic acid and β-sitosterol isolated from the bark, leaves and seeds are proved to be Anti-cariogenic activity.
7. The Zones of inhibitions for the antibacterial activity were compared with the standard as cholrhexidine. The prepared ethanolic extracts of *M. elengi* showed antimicrobial activity against *S. mutans and S. aureus*. The ethanolic extract of *M. elengi* plants parts like bark, leaves and seeds was dissolved in water and DMSO (dimethyl sulfoxide) showed better activity against *S. mutans and S. aureus.* bark was found more potent as compared to leaves and seeds.
8. The zone of inhibition for ethanolic extract of *M. elengi* plant parts like bark, leaves and seeds showed activity against Plaques.
9. Further research is needed to isolate and characterize the novel compounds present in these extracts which can help to formulate effective dosage forms to combat this disease and combination of all above extract are increasing potency give good activity against cariogenic bacteria.

References

1. Prakash Chandra Gupta, *Mimusops elengi* Linn. (Bakul) - A Potential Medicinal Plant: A Review, International Journal of Pharmaceutical and Phyto pharmacological Research, Department of Zoology, Kashi Naresh Government P. G. College, Gyanpur, S. R. N. - 221304, Bhadohi (U. P.), India. 2013; 2(5):332-339.
2. Reem Asrivastava, Gaurishukla Sakshisharma. phyto medicinal importance of *Mimusops elengi:* an emerging present and promising future, Innoriginal International Journal of Sciences, 2017, 4(1).
3. Kanchan Lata Singh, Priya Srivastava, Shiv Kumar. Singh DK, Singh VK, *Mimusops elengi* Linn. (Maulsari): a potential medicinal plant, Archives of Biomedical Sciences. 2014; 2(1):18-29.
4. Pradeep Kumar R. Ethno medicinal plants used for oral health care in India. International Journal of Herbal Medicine. 2014; 2(1):81-87.
5. Reenu Yadav, Dr. SK Yadav. Asian J Pharm Clin Res, Suppl. 2013; 6:16-20.
6. Ankur Kulkarni A, Rahul Deshpande R, Priya Panvalkar, Priyanka Mahajan, Asha Kale, Anjali Ruikar *et al* Comparative evaluation of antibacterial properties of different extracts of *Mimusops elengi* (bakul) & *Juglansregia* (walnut) against salivary microflora, Research Journal of Pharmaceutical, Biological and Chemical Sciences, July–September RJPBCS. 2011; 2(3):636.
7. NCJ Packia, Lekshmi N, Sowmia S. Viveka J, Raja Brindha, Jeeva S *et al* The inhibiting effect of *Azadirachta indica* against dental pathogens, Pelagia Research Library, 2012; 2(1):6-10 .
8. Mounyr Balouiri N, Moulay Sadiki, Saad Koraichi Ibnsouda. Methods for *in vitro* evaluating antimicrobial activity: A review, Journal of Pharmaceutical Analysis, Journal of Pharmaceutical Analysis. 2016; 6:71-79.
9. Shyla Jebashree H, Jayasurya Kingsley S, Emmanuel Sathish S, Devapriya D. Antimicrobial activity of few medicinal plants against clinical isolated human cariogenic pathogens—an *in vitro* study. International scholarly research network isrn dentistry volume, article in 541421, 6 pages doi:10.5402/2011/541421, 2011.
10. WHO. Quality control method for medicinal plant materials. Geneva: WHO, 2002.
11. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 2007 17:149-146.
12. Zhitaoliang, Zhihongjiang, davidwangfunfong and zhongzhenzhao. Determination of Oleanolic Acid and Ursolic Acid in *Oldenlandia diffusa* and Its Substitute Using High Performance Liquid Chromatography, Journal of Food and Drug Analysis. 2009; 17(2):69-77.
13. Vidya Dighe, Dhanashri Mestry. High performance liquid chormatographic method for simultaneous quantitation of betulinic acid and ursolic form dried stem bark powder of *Mimusops elengi* Linn. Int. J Res. Ayurveda Pharm, 2013, 4(6).
14. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW *et al.* High molecular weight plant polyphenolics (tannins) as biological antioxidants, Journal of Agricultural and Food Chemistry. 1998; 46:887-1892.
15. Hagerman A, Harvey-Muller I, Makkar HPS. Quantification of tannins in tree foliage – α laboratory manual .Vienna: FAO/IAEA, 2000, 4-7.
16. Wagner H, Bladt S, Plant Drug Analysis, 2Nd edition, Springer, Berlin, 1996, 230-231.
17. Desai JV, Thomas R, Kamat SD, Kamat DV. Microwave Assisted Extraction of Saponins from *Centella asiatica* & its *in vitro* Anti-Inflammatory Study, Asian Journal of Biochemical and Pharmaceutical Research. 2011; 4(1):331.
18. Sadashivam S, Manickam A, Biochemical method, New Age International (P) Limited, Publisher, 1996 2,195.
19. Zak Dickeaa, Wbite RC, Burnett EG, chedey. Rapid estimation of free and total cholesterol. Amer. I. pat/toṛ. 1954; 24:1307-1315.
20. Vijay D Tambe, Rajendra S Bhambar. Estimation of Total Phenol, Tannin, Alkaloid and Flavonoid in

Hibiscus tiliaceus Linn. Wood Extracts. Research & Reviews: Journal of Pharmacognosy and Phytochemistr, 2014.
21. Rakesh Shivatare S, Ramesh Deoda S, Prasad Kadam V, Hanumant Bhusnar U, Nupura Narappanawar, Manohar Patil J. Pharmacognostic Standards for *Mimusops elengi* Linn - A Review, Journal of Pharmacognosy and Phytochemistry. 2013; 2(3):12-18.
22. Bijayanta Sircar, Shyamapada Mandal. Antibacterial Activity of *Mimusops elengi* Leaf, Seed and Bark Extracts Alone and in Combination with Antibiotics against Human Pathogenic Bacteria. Mandal and Sircar, Transl Med (Sunnyvale). 2016; 6:4.