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RESEARCH ARTICLE

PHYTOCHEMICAL AND ANTIMICROBIAL EFFECT OF PSIDIUM GUAJAVA AND LEUCAS ASPERA AGAINST DENTAL CARIES PATHOGEN

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Abstract

In the present study, the effect aqueous *Psidium guajava* and *Leucas aspera* extract on micro organism causing dental caries infection was investigated. To find out Phytochemical constituent. Analyse the functional group using FTIR spectroscopy. Isolation of micro organism from infected person. Analyse the effect of *Psidium guajava* and *Leucas aspera* extract: on micro organism causing dental caries.

Introduction

During the past decades the common consensus from many reports worldwide was that dental caries had declined significantly and was continuing to decline in populations. The dental community has prided itself on efforts that have reduced dental caries including use of systemic and topical fluorides, toothpastes, sealants, improvements in diet, oral health education and dental care [4,7]. Dental caries is the dissolution of the enamel and dentin in pits, fissures, and interdental regions of the teeth, eventually spreading to buccal and lingual surfaces. Since the release of "Oral Health in America: A Report of the Surgeon General" in May 2000, efforts have not advanced dental caries prevention, its risk of development, or its early detection [6]. Worse, the severity of caries has since been increasing in all socioeconomic groups [7, 5]

Material and Methods

Sterilization

It is a process to achieve sterility. In absolute terms it is referred as the absence of all microorganisms. The procedure was applied of all the media, reagents etc, which are used in laboratory practice.

Gram staining

Crystal violet solution

Dissolve crystal violet in ethanol and the ammonium oxalate in distilled water. Mix the solution A and B obtain the crystal violet staining reagent.

Mordant:

Grind the iodine and potassium iodine and potassium iodide in a mortar and pestel add water slowly with continuous grinding until the iodine was dissolved. Stored in Amber coloured bottles.

Decolourizing agent

Smears were made in clean slides. It was heat fixed before staining process.Staining was done by flooding the smears with crystal violet solution (for 1min) and the smear was washed in a gentle and direct stream of tap water for 2 seconds and flooded the slide with iodine mordant for 1 min after washing with distilled water.

Then it was decolorized with 95% alcohol until no more dyes flows a ways from the samer. some stained cells and other resist depolarization, then it was rinsed with tap water and the counter stain safranin was added, left it for about 30 seconds. It was again rinsed in tap water and dried in air, then it was observed under the oil immersion objective. The gram negative bacteria appear in pink colour, while gram positive cells appear purple. Examination of gram stained organisms usually provides a starting point for classifying identifying and characterizing bacteria. The gram stain of clinical material taken directly from an infection patient could rapidly provide valuable information about the microorganisms causing the disease. This information helps and guides the selection of subsequent tasks needed to identify the bacteria.

Preparation of media.

Media is defined as the substrate in which microorganisms could grow and multiply. For the present study, synthetic media was used for culturing biochemical tests and antibiotic sensitivity purposes. Synthetic media was one in which all the constituents are chemically defined. They are generally used to study the specific nutritional requirements of different microbes.

Blood agar

Blood agar was used to grow a wide range of pathogens. It was also required to detect and differentiate haemolytic bacteria. Twenty eight gram nutrient agar was suspended in 1000ml of distilled water. It was then boiled to dissolved the medium completely and sterilized by autoclaving at 15 Ibs pressure(121^0c) for 15min.cooled to 45-50^0c and 5%sterile defibrinated blood aseptically and mixed well.

Cultural examination

Once the growth was found the primary plate, then the identification was carried out by the following systematic method, for examining type of the colony colour change of the mrdium, the morphology of the cells under stained and unstained conditions and biochemical colony was done by using Bergey's manual of determinative bacteriology (

Oxidase test

During aerobic respiration, oxidase enzyme play a vital role in the operation of electron transport system.cytochrome oxidase catalyzes the oxidation of a reduced cytochrome by molecular oxygen, result in the formation of water or hydrogen peroxide. The enzyme will catalyze the transport of electrons between electron donors in bacteria and a redox dye tetraethyl-p-phenylene-diamine. The dye is reducer to a deep purple colour.

Catalase test

During respiration microorganisms produce hydrogen peroxide and in some instance,an extremely toxic super oxide. Accumulation of hydrogen peroxide and superoxide leads to the death of the organisms unless they are degraded enzymatically. Catalase production should be determined by adding the suitable hydrogen peroxide to an approximately incubated bubbles of free oxygen gas.

Preparation of plant extract

Continuous hot extraction – using Soxhlet apparatus

Soxhlet apparatus is used to execute continuous hot extraction. The soxhlet apparatus is for the extraction of coarse powder and is placed in a " thimble" made filter $_{P}$H :7.4inserted into the wide tabe of the extractor. The solvent, which is taken in the flask,is heated ; the vapour, which arise from the solvent, get into the condenser through a side tube and the liquid condensed from the vapors drips into the thimble. The solvent liquid level slowly rises and during this period, the dried materials get extracted of its soluble constituent. When the level of the liquid reaches the top of the siphon, it gets siphoned into the flask. The suction effect of the siphoning assists permeation of the solvent through the drugs again, a portion the solvent from the solution is vaporized leaving constituents in the flask itself and the process is repeated again and again; this kind of continuents are not readily in the cold and thermobile.

Disc diffusion method

The disc diffusion method provided a simple and reliable test in routine clinical bacteriology in order to find out the effect of a particular substance on a specific bacterium. This method consists of impregnating small circular disc of standard filter paper with given amount of a chose concentration of substance.

Preparation of the disc

Disc usually consist of absorbent paper impregnated with antibacterial agent It was most convenient to use Whatman No.1 filter papar for preparing the disc .Dry discs of 6mm diameter were prepare and sterilized in autoclave.

Muller hinton agar

Thirty eight of muller hinton agar was suspended in 1000ml of distilled water and was boiled to dissolve the medium completely and dispensed in 18ml quantities in universal containers and sterilized by Autoclaving 121^0c for 15min.

Phytochemical:

Test for alkaloids

A small portion of the extract was stirred with a few drops of dilute hydrochloric acid and filtered.

Test for carbohydrates and glycosides

The minimum amount of extracts were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates and glycosides.

Molisch's test

The filtrate was treated with 2-3drops of 1% alcoholic alpha naphthol, and 2ml of concentrated sulphuric acid was added along the sides of the test tube violet color was obtained.

Fehling's test

The filtrate was treated with 1ml of fehling's solution and heated. A reddish orange precipitate was obtained.

Test for glycocides

Another portion of the extract was hydrolyzed with hydrochrochloric acid for a few hours on a water bath and the hydrolysate was subjected to legal's,Borntrager's test to detect the presence of different glycosides.

Legal's test

To the hydrolysate 1ml of sodium nitroprusside solution was added and then it was made alkaline with sodium hydroxidesolution. None of the extracts produced pink to red colour.

Borntrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. No color change in Ammoniacal layer was observed.

Test for Phytosterol

1gram of the extract was dissolved in few drops of dilute acetic acid,3ml of acetic anhydride was added followed by few drops of con.sulphuric acid .Appearance of bluish green colour shows the presence of phytosterol.

Test for fixed oils and fats

a. Small quantity of the various extracts was separately pressed between two filter paper.oil stain on the paper indicates the presence of fixed oil .
b. Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on water bath for 1-2hrs.Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

Test for saponins

The extract was diluted with 20ml of distilled water and it was agitated on a graduated cylinder for 15min.The presence of saponins was indicated by formation of 1cm layer of foam.

Test for tannins and phenolic compounds

Small quantities of various extracts were taken separately in water and tested for the presence of the presence of phenolic compounds and tannins with

(i)Dilute ferric chloride solution (5%)- Violet colour

(ii) 1% solution of gelatin containing 10%NaCl- White precipitate.

(iii) 10% lead acetate solution- White precipitate

Test for proteins and free aminoacid

Dissolved small quantities of various extracts in a few ml of water and treated with

(i) Millon's reagent – Red colour

(ii) Ninhydrin reagent – purple colour

(iii) Biurett test –Equal volume of 5% solution and 1% copper sulphate solution were added.pink or purple colour.

Test for gums and mucilages

About 10ml of the extract was added to 25ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Test for flavonoids

Shinoda's test

To the extract, magnesium turnings and few drops of concentrated hydrochloride acid were added and boiled for 5min.Red color was obtained

Result and Discussion

The dental caries is an ecological disease in which the diet, the host and the microbial flora interact over a period of time in such a way as to encourage demineralization of the tooth enamel with resultant caries formation.Dental caries is still one of the most common disease in the world today.Until recently almost everyone had experienced

tooth decay in their lifetime.The bacterial infection affects humans throughout their life. The infection depends on the nature of the organisms and interacts with the human body. The present study, the dental caries samples were collected. The identified pathogens were *Streptococcus mutans and Streptococcus mitis* He was isolated it form a carious lesion but it was not until the 1960s that real interest in this microbes was generated when researchers began studying dental caries in earnest. The *Streptococcus mutans* isolate were sub-divided into a number of distinct species some of which were of animal and some human sources. . Thus the "*Mutans Streptococcus*" were born and the name *Streptococcus mutans* was retained to describe the most common of the two main human strains. The present investigation is also in agreement with the previous report showed the zone of inhibition in Snuff powder and *Chebulic myrobalan* against the *Streptococcus* species more over the result revere the degree of sensitivity depend upon the concentration o the extract. The antibacterial activity of leaf extract *Psidium guajava* against the bacteria such as *Streptococcus mutans and Streptococcus mitis* was studied. The antibacterial efficacy was determined by the standard well diffusion method. The *Psidium guajava* extract showed greater activity against *Streptococcus mutans and Streptococcus mitis 50* µg concentration .The antibacterial activity of *Leucas aspera* extracts against dental caries causing bacteria such as *Streptococcus mutans and Streptococcus mitis* was studied. The *Leucas aspera* extract *have maximum inhibitrion zone at 50* µg concentration *was obtained when compared to Psidium guajava extract.* Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes.Above results lead to the conclusion the data was consistent with the hypothesis, the diameter of zone of inhibition obtained from the observed data showed the similarities with experimental data.

Phytochemical investigation of *Psidium guajava* and *Leucas aspera* extract:

The preliminary phytochemical analysis was carried out in the leaf extracts of *Psidium guajava* and *Leucas aspera* extract. The leaf extracts *Leucas aspera* showed an indication of the presence of as alkaloids, reducing sugar, phenolic compound, and flavonoid were confirmed in suitable chemical test..The extract of *Psidium guajava cotain* alkaloids, phenolic compound, and tannin.(Table-5a-b,plate-5) Phytochemical analysis of test plant showed presence of biologically active compounds such as phenols and tannins in extracts. These organic compounds have been known posses antimicrobial activity. Many disinfectant used in the hospital such as Lysol, cresol and dettol containing phenol as the active ingredients. Plants contains a spectrum of secondary metabolites such as phenol, coumarins, flavaonoids, quinines, tannins and the glycosides, alkaloids and essential oils etc., The study plant also posses oils are coinciding with result of above workers. Plant contains a spectrum of secondary metabolites Such as alkaloids, phenols, flavonoids, quinines and their glycosides, quinones and essential oil. Workers. The test plants of this present study also has rich in flavonoids, alkaloids, steroids and glcosides flavour the antibiotic potentials. Phytochemical analysis of test plant showed presence of biologically active compounds such as phenols and tannins in extracts. These organic compounds have been known posses antimicrobial activity. Tannins were found to be a component of plants that showed antibacterial activity. Natural products are rich sources of bioactive compounds, and play an important role in the development of new drugs. Between 1981 and 2002, 62% of the new and approved drugs for cancer were compounds with natural origins. For infectious diseases, the number is still higher, at 75%. As for anti-inflamatory drugs, 26% were natural product-derived compounds. Overall, 67% of the 1031 new drugs approved for all diseases during this time period were compounds that were not of purely synthetic origin. The phytochemical study and antifungal screening of the leaf extract of Eclipta Prostrata and Phyllanthus Niruri was carried out in the present study. The present study using the organic solvent exctraction technique the bioactive constituents of the Snuff powder and Chebulic myrobalan have been exctracted and subjected to preliminary colour test to identify the nature of the compounds such as Alkaloids, Reducing sugar, Phytosterol, Fixed oil and fats, Phenolic compounds tannis ,Gums and Muscilage were confirmed by suitable chemical test

TABLE- 1: **Biochemical test for the identification of pathogenic Microorganisms**

S.NO	Test organisms	Biochemical test			
		Colony morphology	Gram staining	Motility	Spore formation

1.	*Streptococcus Mutans*	rough	Positive coccus	Non-motility	Non-spore
2.	*Streptococcus Mitis*	soft	Positive coccus	Non-motility	Non-spore

b) PRELIMINARY PHYTOCHEMICA SCREENING OF *leucas aspera*

Table-2

S.NO	**TEST**	***Leucas aspera***
1	Alkaloids	+
2	Terpenoids	_
3	Steroids	_
4	Coumarins	_
5	Tannins	+
6	Flavinoids	+
7	Phenols	+
8	Volatile oils	_
9	Quinone's	_
10	Sugar	+

TABLE-3: IDENTIFICATION OF COMPOUNDS FROM psium guvajava USING FTIR TECHNIQUES;

s.no	Frequency range	Wavelength range	type and group
1	3637.29	2.72-3.12	silicon,boron compounds
2	3195.15	2.98-3.11	Cis form

3.	2075.07	4.61-4.81	Azides
4	1639.73	6.06-6.35	Pyridines
5	690.17	14.49-16.26	Cis form

TABLE-4

IDENTIFICATION OF COMPOUNDS FROM *leucas aspera* USING IR TECHNIQUES

s.no	Frequency lrange	Wavelength range	Type and group
1	3929.61	2.34-2.39	oximes
2	3816.84	2.45-2.48	ketones
3	3418.00	2.89-2.91	sec. amides, free NH
4	2913.87	3.40-3.43	Alkanes
5	2154.35	4.64-4.69	Carbodumides
6	1642.80	5.95-6.05	trans form
7	1566.26	6.37-6.67	all amino acids
8	1420.22	7.00-7.41	Sulfites
9	1159.37	8.70-9.35	Aliphatic ethers
10	1049.90	9.52-10.1	phosphorus compound
11	618.94	15.90	Alkynes

TABLE-5

Antibacterial screening of leaf extract of *Psidium guajava* and *Leucas aspera* on *Streptococcus mutans*

S.NO	**Antibacterial agent**	**µg**	**Zone of inhibition in diameter (mm)**	
			Standared	**Observed**
1	*Psidium guajava*	25	27	14
		50	27	22
		25	27	16

2	*Leucas aspera*	50	27	25

square value significance at 5% level

TABLE-6

Antibacterial screening of leaf extract of *Psidium guajava* and *Leucas aspera* on *Streptococcus mitis*

S.NO	Antibacterial agent	Mg	Zone of inhibition in diameter (mm)	
			Standared	Observed
1	*Psidium guajava*	25	27	11
		50	27	20
2	*Leucas aspera*	25	27	13
		50	27	25

square value significance at 5% level

Summary and Conclusion

Herbal medicine represents one of the most important fields of traditional medicine. The medicinal plants played an important role in the health care of about 80% of the world population in developing countries and depend largely on traditional medicine.The Present investigation carried out that Preliminary Phytochemical analysis were studied for aqueous extract of *Psidium guajava* and *Leucas aspera* .

The extract of *Leucas aspera* possess more phytocompound when compared to *Psidium guajavaextract.* The functional groups were analyzed by FTIR spectrum.The results of various screening tests indicate that the herbal plants possess some measurable inhibitory action against the dental decaying *Streptococcus* species.The present work concludes that the extract of *Psidium guajava* and *Leucas aspera* more suitable to inhibit the *Streptococcus* species. From the above premliminary study, we conclude that the ethanolic extract of *Psidium guajava* and *Leucas aspera* proved to be one of the herbal remedies for dental decay organisms.We recommend that the *Leucas aspera* is more suitable herbal powder to inhibit decaying organisms. So that it can be used as a potential source for the development of a phytomedicine to act against dental caries causing bacteria.

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