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Research Article

Study on the Dental Caries Prevention Using Traditional Medicinal Plants of Bangladesh

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Abstract

Crude rectified spirit extract of fifteen (15) selected medicinal plants under investigations were screened against dental caries forming bacteria from the 24 patients of Rajshahi Medical College Hospital. Preliminary screening reveals that only eleven plant extracts showed moderate to strong activity against cariogenic bacteria. Second screening leads to select only three (3) plants for the highest activity against cariogenic bacteria. These are *Allium sativum, Psidium guajava* and *Erythrina variegata*.

Chloroform fraction of the crude rectified extracts of the *Allium sativum* showed the highest activity against cariogenic bacteria *Streptococcus mutans* both at a concentration of 200 and 400 µg/ml. Column chromatographic separation of this fraction a mixture of two compounds with very close RF values were isolated and identified as a mixture of Allicin and Allin having very strong activity against *S. mutans* with a zone of inhibition 19 and 24 mm at a concentration of 200 and 400 µg/ml disc of the compound which is somehow stronger than the standard kanamycin (11 mm, 30 µg/disc). A compound PG-1 was isolated from ethyl acetate extract of *P. guajava* showed remarkable, activity against *S. mutans* both at a concentration of 200 and 400 µg/ml(zone of inhibition 18 and 24mm). The compound was identified as Pinfaencin by spectral analysis.

β-Sitosterol was also isolated from the methanol fraction of rectified spirit extract of the plant *E. variegate* showed moderate activity against cariogenic bacteria *Streptococcus mutans*, both under similar concentrations.

Keywords: Dental Caries; Cariogenic Bacteria; Medicinal Plants; Permanent Teeth; Compounds

Abbreviation

SA-1: Allium sativum-1; PG-1: Psidium guajava-1; EV-1: Erythrina variegate-1

Introduction

Dental caries is a progressive irreversible bacterial damage to the tooth tissues. One of the most major causes of all the diseases

is tooth loss. It is a biofilm-related oral disease, which continues to afflict the majority of the World's population. The disease results from the interaction of specific bacteria with constituents of the diet within a biofilm formed on the tooth surface clinically known as dental plaque. Although additional microorganisms may be also involved, *Streptococcus mutans* plays a key role in the pathogenesis of the disease. This bacterium is able to: (i) produce and tolerate acids; (ii) synthesize water-insoluble glucan from sucrose through the activity of glucosyl transferases (GTFs); and (iii) adhere tenaciously to acquire pellicle on tooth surfaces [1,2]. The combination of these virulence properties allows *S. mutans* to effectively colonize tooth surfaces and modulate the transition of nonpathogenic to highly cariogenic dental biofilms, which leads to caries formation (Sharma and Joshi. 2008). Therefore, approaches aimed to inhibit the viability and virulence properties of *S. mutans* could be precise and selective for prevention of dental caries. *Dryopteris crassirhizoma* is a semi-evergreen plant that grows on the deciduous forest floor as a pteridophyte [3]. The plant is widely distributed in Korea, China and Japan, and the roots are traditionally used as an herbal remedy for various diseases, such as tape worm infestation, the common cold and cancer [4]. Many studies of the plant have revealed numerous pharmacological properties, including antioxidant, anti-cancer and anti-bacterial activities [3,4]. Furthermore, a study showed the potential of the plant as an agent for the prevention of oral diseases, such as dental caries [5]. Medicinal plants are useful in many diseases dates back from the history. Plants like *Acacia leucopholea, Albizzia lebback, Bridalia grandis, Drosera peltata, Erthrina variegate* and many other selective plants active against *Streptococcus mutans, Streptococcus sobrinus, Streptococcus oralis, and lactobacillus* species has been listed and this review highlight the role of medicinal plants and phytochemicals like flavonoids, polyphenols, terpenes, alkaloids in the treatment of dental caries and infections associated with dental care [6]. Generally, the pharmacological activities of the plant have been attributed mainly to the presence of triterpene, phloroglucinol, flavonoid and other phenolic analogs [7]. Medicinal plants have been used as a major source of innovative and effective therapeutic agents throughout human history and have shown promise as a source of components for the development of new drugs [8]. In addition, studies using medicinal plants to prevent or treatment of oral diseases such as dental caries have received a great deal of attention because the use of commercial chemotherapeutic anti-caries agents, such as chlorhexidine and triclosan, not only remains controversial but also has common side effects including tooth staining and the emergence of bacterial resistance. Recently, several studies have shown the feasibility of using medicinal plants as a source of chemotherapeutic agents for the prevention of oral diseases, particularly dental plaque-related diseases [4,5,9]. A number of compounds, such as epicatechin, allicin and sanguinarine, isolated from medicinal plants, have also been investigated for their efficacy against oral microbial pathogens [10,11].

Medicinal plants are an essential constituent of the indigenous medical systems around the world. These resources are part of their traditional knowledge. As a part of our ongoing research program on phytochemical investigation of medicinal plants of Bangladesh, we recently focused on *Erythrina* species, *Allium sativum and Psidium guajava* and have reported bioactive secondary metabolites from *Erythrina caffra*, *Erythrina lysistemon*, and *Erythrina latissima* to name a few.

Objectives

General objectives

To find out the effects of traditional medicinal plants for dental caries prevention

Specific objectives

Present paper consists of two parts

- The Biological investigation which included:
- To collect the dental caries from the affected teeth.
- To assess the types of bacteria.
- To identify the family of responsible bacteria.
- To culture the bacterial strains.
- To calculate the number of family identified bacteria.
- To determine the number of sensitivity of bacteria.
- Collection of the plants to use against bacteria responsible for dental caries.

Chemical investigation which included the following

- Isolation of the active compound.
- Structure determination of isolated compounds.

Materials and Methods

Biological work

General methodology

The method used in the present work is to isolate cariogenic bacteria from the dental caries bearing patients and tested against rectified sprit extract of the selected medicinal plants/plant parts for their antibacterial activity. *Streptococcus mutans* is the major cause of dental caries in the patients both adults and children. Isolation of *S. mutans* after culture and sub-culture were subjected to test against selected plant extracts. The extracts which showed strong activity against *S. mutans* were used for further work.

Selection of medicinal plants

Present investigation aimed to record folk medicinal use of plants for oral health care by conducting a survey in rural and urban areas adjoining areas of Rajshahi University campus. Ethno botanical survey with respect to use local medicinal plants by urban people for management of oral problems was carried out during the period of July-2013 to June 2014. Present investigation reveals that 21 ethno botanical plant species belonging to 21 families are being used for oral health care in the form of tooth brush (tender branches), Tooth powder (ground plant parts) or charcoal powder.

Of the 21 medicinal plants used for dental care, 15 have been selected for the study and are cited in Table-1(Result and discussion section).

Selection of patients having dental caries

Dental carries are common in rural areas in both children and adults permanent teeth. During the tenure of the research work 452 patients bearing dental caries both male and female were identified. Cariogenic bacteria from 311 male and 231 female patients were isolated and characterized for their antibiogram. Two Gram positive coccal forms of bacteria *L. acidophilus* and *S. mutans* have been implicated in dental caries from over a century.

The *streptococcus* bacteria along with other bacteria causing dental caries were isolated from the samples of 24 patients (among 452 patients) having sevier dental caries and tested against the above 15 selected medicinal plants for their activity against *S. mutans* bacteria (Table-2, results and discussion section).

Collection of caries specimens

- Sample: Caries infected teeth
- Study site: Dental Unit of Rajshahi Medical college Hospital.
- Study place: Molecular Biology lab. IBSc, RU.

In vitro antibacterial screening

In vitro antibacterial screening of the crude extracts or pure compounds can be measured by examining the growth response of various microorganisms which were placed in contact with them. In the present study, the disc diffusion method was used. In this method dried and sterilized filter paper discs (4-6mm in diameter) containing the test samples of known concentration were placed on the Agar media contained in petry dish. The petry dishes were kept at a low temperature of 4^0 C for 24 hrs. and were then incubated at a temperature of 37^0 C and kept for 24 hrs. to allow optimal growth of microorganism.

If any test sample has antibacterial activity it will inhibit the growth of microorganism in the media surrounding the disc is created. This clear zone is known as zone of inhibition and the diameter of zone of inhibition is measured in terms of millimeter (mm). The larger the diameter of zone of inhibition, the greater the activity of the test sample against that particular bacterium. Nutrient agar media is used for antibacterial activity test.

In preliminary screening rectified sprit extract of 15 plants or plant parts were tested against dental caries bacteria isolated from 24 patients. Screening result is shown in Table-2 (Results and discussion section). Throughout the entire work antibiotic Kanamycin was used as standard and a blank experiment was performed.

Phytochemical work

The collected bulb and bark of the three plants, *Allium sativum, Psidium guajava, Erythrina variegate* each (500gms) were dried and pulverized. Extractions of each of the plant was done with rectified sprit (3 L) in a Soxhlet apparatus at its boiling point, filtered and concentrated under reduced pressure to obtain semi-solid masses 17.2, 14.6 and 16.5gms, respectively. All these three crude extracts of the above plants were partitioned with *n*-hexane, chloroform, *n*-hexane, ethyl acetate, and *n*-hexane, ethyl acetate and finally with methanol, respectively. Evaporation of the solvent under reduced pressure to afford the respective fractions. Column chromatography of the chloroform soluble fraction and preparative TLC of the column fraction, eluted with *n*-hexane: chloroform (4:1) from the plant *Allium sativum* afforded a mixture of two compounds with very close R_f values (2.4gm) and were not separable.

Column chromatography of the ethyl acetate soluble fraction of the plant extract of *Psidium guajava* afforded a compound PG-1 as an amorphous powder (83.0 mg) from the fraction of the column eluted with *n*-hexane: ethyl acetate (20:1 and 10:3) after workup, purification and crystallization.

After column chromatography of the ethyl acetate soluble fraction of the plant *Erythrina variegate,* a compound EV-1 was obtained (0.93gm) as an amorphous powder from the column eluted with ethyl acetate: methanol (10:1, and 5:1) fractions.

All three compound/compound mixture SA-1, PG-1 and EV-1 showed moderate to strong activity against dental caries forming bacteria *S. mutans* at a dose of 100 to 400 mg and were as such subjected to spectral analysis for structure elucidation.

Results and Discussions

Microbiological Investigation

One of the most important and effective *in vitro* antibacterial screening of the crude extract or pure compound is the disc diffusion method. In this method dried and sterilized filter paper disc (4-6mm in diameter) containing the test samples of known concentration were placed on the agar media contained in petry dish. The petry dishes were kept at a low temperature of 4^0C for 24 hrs. and were then incubated at a temperature of 37^0C and kept for 24 hrs. to allow optimal growth of micro-organism. For preliminary screening to see only the activities of the plant extracts no standard antibiotic was used and no blank experiment was performed. If any test sample has antibacterial activity it will inhibit the growth of microorganism in the media surrounding the disc is created. This clear zone is known as zone of inhibition and the diameter of zone of inhibition is measured in terms of millimeter (mm). The larger the diameter of zone of inhibition, the greater the activity of the test sample against that particular bacterium. Nutrient agar media is used for antibacterial activity test.

For preliminary screening, the *Streptococcus mutans* bacteria along with others causing dental caries, were isolated from the sample of 24 patients having dental caries and tested against the rectified spirit extracts of 15 plants or plant parts for their antibacterial activity using disc diffusion method (Table 1). 11 plants showed moderate to strong anti-microbial activity and are given in table 2.

Specimen (Plant)sample Patient sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	na	10	11	13	na	na	11	11	14	10	11	10	11	na	9
2	na	12	13	13	na	na	12	14	16	15	14	10	13	na	10
3	na	10	12	12	na	na	12	14	15	13	13	11	12	9	Na
4	na	14	15	13	na	na	14	16	15	11	14	13	15	na	9
5	na	16	17	15	na	na	15	20	18	15	16	13	15	na	11
6	na	20	17	15	na	na	18	19	20	15	14	12	16	na	11
7	na	20	17	18	na	na	20	18	19	15	13	11	22	na	11
8	na	12	13	10	na	na	12	12	10	12	11	11	18	na	10
9	na	12	13	11	na	na	12	14	14	10	13	12	13	na	11
10	na	13	12	12	na	na	13	12	13	10	14	12	15	9	Na
11	na	15	15	13	na	na	14	14	16	14	16	13	17	na	Na
12	na	15	11	11	na	na	14	13	12	11	10	12	13	na	8
13	na	10	18	14	na	na	10	15	14	11	15	13	12	na	11
14	na	10	12	10	na	na	11	16	15	13	12	11	11	na	10
15	na	15	14	12	na	na	16	10	18	14	14	11	13	na	10
16	na	12	10	12	na	na	12	11	14	10	12	11	11	na	11
17	na	11	12	12	na	na	11	11	12	11	13	12	14	na	10
18	na	11	13	12	na	na	12	12	13	11	13	10	14	na	10
19	na	09	11	10	na	na	09	14	14	13	16	11	14	na	09
20	na	10	10	12	na	na	10	12	13	10	14	11	13	na	Na
21	na	12	15	12	na	na	12	11	13	10	13	13	12	na	Na
22	na	15	11	12	na	na	14	10	12	11	10	11	11	na	8
23	na	15	18	14	na	na	14	17	11	10	16	13	15	na	11
24	na	14	12	10	na	na	15	16	24	10	11	13	12	na	9

Table 1: Preliminary antibacterial assay/anti-bacterial sensitivity test result (1 mg/disc): Zone of Inhibition in (mm).

Sl. No	Specimen code	Scientific name	Local Name	Using part
01	10	*Erythrina variegata Linn*	Mandar	Bark
02	7	*Allium sativum*	Garlic/ rasun	Bulb and Seed
03	12	*Psidium guajava*	Guava/ Peyara	Bark
04	13	*Swertia chirayta*	Chirata	Whole Plant
05	9	*Morus alba Linn*	Tut	Bark
06	11	*Terminalia chebula*	Haritaki	Seed
07	8	*Syzygium aromaticum*	Clove/Lobongo	Flower Stalks
08	4	*Croton tiglium*	Jamalgota	Stem
09	3	*Azadirachta indica*	Neem	Bark
10	2	*Mimusops elengi*	Bokul	Bark
11	15	*Andrographis paniculata*	KaloMegh	Whole Plant

Table.2: Plant specimen showing antibacterial sensitivity (according to the chronology of sensitivity).

Out of these 11 plants 3 plants *Allium sativum*, *Psidium guajava* and *Erythrina variegata* were selected for their highest antimicrobial activity.

Phytochemical works

Allium sativum (Garlic)

The bulb extract of *Allium sativam* showed the highest zone of inhibition (20 mm) at a dose of 1mg/disc against dental caries forming bacteria isolated from 5 patients (Table 3) and hence strong activity. Similar two experiments were also performed for clarification with a dose of 2 and 3 mg/disc and the results showed in a dose dependent manner.

Next work is to isolate the active compound from the above selected three plants which will show highest activity against the isolated pathogenic bacteria and the structures of the isolated compounds will be determined using spectroscopic methods of analysis.

Specimen (Plant) sample / Patient sample	7. *Allium sativum* Zone of inhibition (mm)	10. *Erythrina variegate* Linn Zone of inhibition (mm)	12, *Psidium guajava* Zone of inhibition (mm)
1	11	10	10
2	14	11	13
3	15	15	13
4	18	15	12
5	20	15	11

Table 3: Selected plants specimen showing antibacterial sensitivity (1 mg/disc).

The crude rectified sprit extract of *Allium sativum* was partitioned with *n*-hexane, chloroform and methanol, after workup, the chloroform fraction showed highest activity against *Streptococcus mutans* (zone of inhibition 14 mm) at a dose of 200 µ gm/disc when tested against four Gram positive and Gram negative bacteria. The chloroform fraction was then subjected to a column of Silica gel eluted with n-hexane, then n-hexane and chloroform with increasing portion of chloroform and finally with chloroform only. The fractions eluted with n-hexane and chloroform (20:1 and 10:1) showed two prominent spot on TLC with a very close R_f values (0.73). After evaporation of the solvent, the isolated compounds were tried to separate using preparative TLC with multiple developments and partial crystallization and it was found these two compounds were un separable. The mixture was designated as Compound SA-1 and was such subjected for chemical and spectral analysis analyses. The compound also showed strong antimicrobial activity against *Streptococcus mutans* (zone of inhibition 19 mm) at a dose of 200 µgm/disc when tested against four Gram positive and Gram negative bacteria which is somehow greater than Kanamycin (30 µgm/disc) used as standard. This is perhaps due to the partial resistant to Kanamycin.

Characterization of compound SA -1

Chemical analysis of the compounds SA-1 showed the presence of an acid group, and gave positive test for unsaturation with bromine water. Moreover, it also gave a positive test for primary amine with alkaline β-napthol solution.

^{1}H-NMR spectral analysis (JEOL-Ex 500 MHz, Ft-NMR Spectrometer) with $CDCl_3$ was done at Strathclyde University, Glasgow, London and TMS was used as an internal slandered and the chemical shift are given in δ value.

Compound mixture SA-1 was also identified as a mixture of two compounds Allicin and Allin and showed a cluster of 9 olefinic peaks between δ 5.00 to δ 5.95 which correspond to 6 allylic protons of Allicin and 3 allylic protons of Allin by comparing chemidraw data of the two compounds. Two methylene proton signals are appeared at δ 3.23 and δ 3.31 both as singlets. Moreover, a methyl group signal appeared at δ 1.51 as singlet corresponding to the methyl proton of Allin. The two protons of primary amine of Allin appeared between δ 2.050and 2.05. All other peaks were not identified because of low resolution and expansion of the spectrum. Comparing the chemidraw spectral data with the 1H-NMR data of SA-I it can be tentatively assigned that the mixture SA-1 composed of two compounds, Allicin and Allin and this mixture showed strong antibacterial activity against major dental caries forming bacteria *S. mutans.* Structures of these two compounds are given below (Figure a and b).

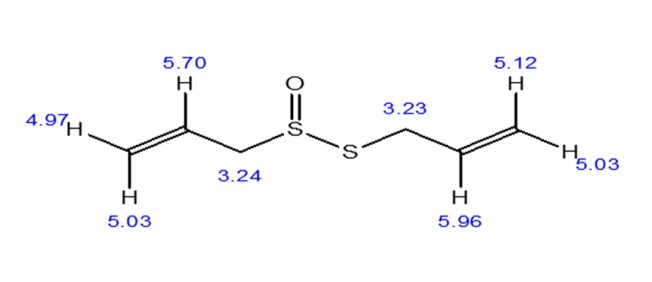

Figure a: Structure of the compound: Allicin.

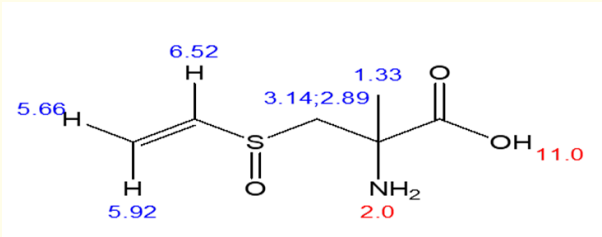

Figure b: Structure of the compound: Allin.

Psidium guajava (Guava)

To the bark extract (14.6gm) of *Psidium guajava* water (50 ml) was added and partitioned with n-hexane (3 times) in a separating funnel. After drying the solvent of the combined organic layers with anhydrous sodium sulphate, was evaporated off under reduced pressure to afford oil (60 mg). The aqueous layer was then again partitioned with chloroform in a similar manner as above to afford chloroform fraction also as oil (1.32gm). After separation from the organic layer the aqueous layer was again extracted with ethyl acetate and usual work up to afford a semisolid mass (11.6gm). *n*-Hexane and chloroform extract did not show any remarkable spot on TLC while ethyl acetate fraction showed one prominent greenish spot.

Ethyl acetate was then subjected to a column of silica gel eluted with n-hexane, n-hexane and ethyl acetate with increasing portion of ethyl acetate, then ethyl acetate and methanol and increasing portions of methanol and finally with methanol. Fractions eluted with n-hexane and ethyl acetate (20:1 and 2:3) showed similar prominent spot-on TLC. Evaporation of the solvent of the combined fraction afforded a compound PG-1 as amorphous powder (83 mg).

Characterization of Compound PG-1

Chemical study of the compound showed the negative test for alkaloid and steroid but showed an intense yellow color with NaOH solution which becomes colorless on addition of a few drop of dilute acid that indicated the presence of flavonoids. Positive test with 2, 4-dinitrophenyl hydrazine reagent indicated the presence of carbonyl functionality in compound PG-1. The compound also showed the positive test for aromatic OH group along with the test for unsaturation with bromine water.

^{1}H-NMR spectral analysis of the compound PG-1 run with 500 MHz instrument (Bruker) using $CDCl_3$ as solvent and TMS as an internal standard was presumed to be identical with pinfaensin a known flavon type compound isolated previously from the plant *Psidium guajava and Rubus pinfaensis* [12].

The structure of the compound is given below

From the chemidraw spectrum of the compound pinfaensin showed a CH_3 group protons as doublet at δ1.30 by coupling with the germinal proton to this CH_3 group at 2 position of the ring.

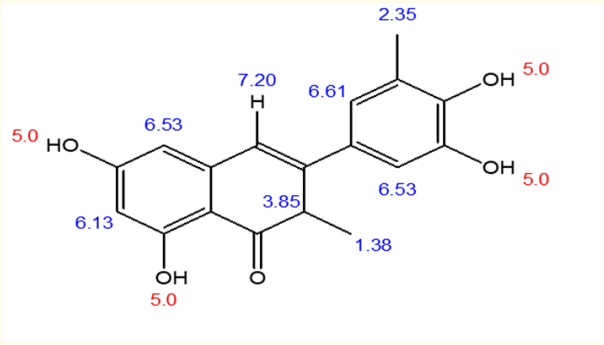

Figure c: Pinfaensin.

Number of protons	Groups of protons	Chemical shift of protons	
		Chemdraw	Isolated compound
2	Me	1.38 (d)	1.30 (3H, bs)
2	H	3.85 (q)	severs small peaks
4	= H	7.20 (s)	7.00
1′	Ar Me	2.35 (s)	3.20 (s)
2′,3′ and 2′′,4′′	Ar OH	5.0 (bs)	5.0 (bs)
4′	Ar H	6.53 (s)	6.45 (s)
6′	Ar H	6.61 (d)	6.60 (s)
1′′	Ar H	6.53 (s)	6.45 (s)
3′′	Ar H	6.13 (s)	6.15 (s)

Table 4: The compound GA-1 was found to be identical with the chemdraw structure.

The geminal H-1 proton signal gives a quartet at δ3.85 (very small peak). Another Ar-Me group protons disposed at 1′ position gives a singlet at δ2.35. Four protons of aromatic OH group give a broad singlet at δ 5.0. An olefinic proton disposed at 4 position of this ring gives a singlet at δ7.20. Four aromatic protons of this compound disposed at 4′, 6′ and 1′′, 3′′ positions with chemical shifts at δ 6.53, 6.61 and 6.53, 6.13, respectively.

The compound GA-1, isolated from *Psidium guajava* (Guava) also showed a broad singlet for CH_3 group instead of a doublet and its germinal proton 2-H did not identified as quartet due to low resolution of the spectrum but several small peaks are appeared there. Aromatic methyl group disposed at 1′ position gives a singlet at δ3.20. Four protons of four aromatic OH groups showed a broad singlet with high intensity at δ5.0. The olefinic proton disposed at 5 position showed a singlet at δ7.0. The rest four aromatic protons at H-4′, H-6′ and H-1′′, H-3′′ showed their presence in the aromatic region at δ6.45, 6.60 and 6.45, 6.15 as singlets, respectively.

From the above analysis and comparing the data (Table-3) of the isolated compound with the chemdraw spectrum, the compound GA-1 was found to be identical with the chemdraw structure (Table 4). Hence it can be concluded that the isolated compound isolated from the bark of *Psidium guajava* is pinfaensin.

Erythrina variegata Linn (Mandar)

To the bark extract (16.5gm) of *Erythrina variegata* water (50 ml) was added and partitioned with n-hexane and ethyl acetate separately (3 times) in a separating funnel. After drying the solvent of the combined organic layers of both extract with anhydrous sodium sulphate and were evaporated off under reduced pressure to afford oily mass 0.3gm and a semisolid mass 2.45gm from both extracts, respectively. After separation from the organic layer the aqueous layer was the dried and extracted (3 times) with methanol. After drying and usual work up methanol extract afforded a semisolid mass (12.66gm). n-Hexane and ethyl acetate fractions did not show any remarkable spots while the methanol extract showed one prominent brown spot with a R_f value of 0.84 with other minor spots on TLC.

A portion (10gm) of this semisolid mass was then subjected to a dry column of silica gel and eluted with ethyl acetate, ethyl acetate and methanol with increasing portion of methanol and finally with methanol only. The fractions eluted with ethyl acetate and methanol (10:1 and 10:2) showed similar single spots on TLC by spraying the chromatogram with vanillin-H_2SO_4 reagent and under UV and with iodine vapor. The solvent of these fractions were combined, dried and evaporated off under reduced pressure to afford white powder which on crystallization gave compound EV-1 (0.59gm) as amorphous powder.

Characterization of compound EV-1

Compound FV-1 is a white amorphous powder gave negative test for alkaloids and flavonoids but showed positive test for steroid with Salkowski and Liebermann-Burchard reagent and test for alcoholic hydroxyl group. Moreover, it also gave a positive test for unsaturation with bromine water.

^{1}H-NMR spectral data are more or less identical with the spectral data of the known steroidal compound β-sitosterol reported in the literature. The spectrum was taken with 500 MHz, spectrometer, $CDCl_3$ solvent and TMS was used as an internal stand.

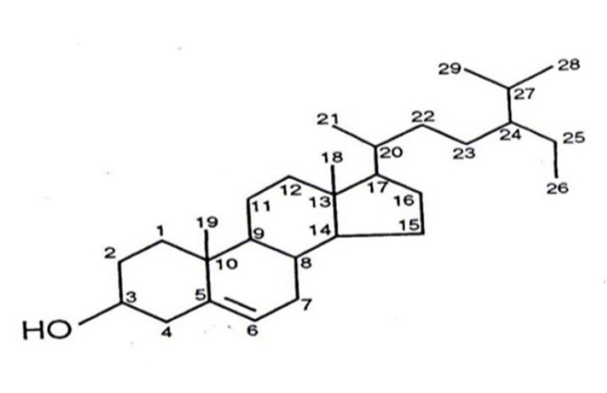

Figure d: β-Sitosterol.

From the ^{1}H-NMR data 8 methyl protons appeared between δ 0.66 to 2.32 all as singlets, H-3 proton geminal to OH group appeared as multiplet between δ 3.3 to 5.28. Moreover, H-6 olefinic proton appeared as broad singlet instead of triplet due to low resolution of the spectrum.

Comparing the ^{13}C-NMR data of β-sitosterol, chemical shifts of all the carbons are almost identical with the literature value as tabulated (Table 5) [13].

Carbon number	Authentic β-sitosterol	Compound
1	37.2	36.9
2	31.5	31.4
3	71.7	71.5
4	42.3	42.3
5	140.7	140.6
6	121.7	121.7
7	31.9	31.9
8	31.9	31.9
9	50.1	50.1
10	36.5	36.6
11	21.1	21.1
12	39.6	39.7
13	42.2	42.2
14	56.8	56.7
15	24.3	24.3
16	28.2	28.1
17	56.1	56.2
18	11.9	11.9
19	19.4	19.4
20	36.1	36.1
21	19.3	19.3
22	33.9	33.8
23	29.1	29.2
24	50.1	50.1
25	26.1	26.1
26	18.7	18.9
27	29.8	29.3
28	23.1	23.1
29	11.8	11.8

Table 5: The ^{13}C-NMR data of compound EV-1 with the authentic sample are given below.

From the above analysis it can be concluded that the isolated compound EV-1 is β-sitosterol having moderate actively against *Streptococcus mutans*.

Conclusion

The antimicrobial compounds from plants extracts may inhibit bacterial growth or kill the bacteria by various mechanisms may have a significant clinical value in the treatment of resistant microbial strains. Recently, traditional medicine has served as an alternative form of health care and to overcome microbial resistance has led the researchers to investigate the antimicrobial activity of medicinal plants.

The method used in the present work is to isolate the cariogenic bacteria from the patients having dental caries and tested against the rectified spirit extracts of the selected plants or plant parts for their antibacterial activity. *Streptococcus mutans* is the major bacteria causing dental caries of the patients. Isolation of *S. mutans* and culture to increase their growth and subjected to test against the selected plant or plant parts having strong antibacterial activ-

ity. Isolation of the bio-active principle was done and structures of the isolated compounds were determined using spectroscopic methods of analysis.

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Conflict of Interest

There is no conflict of interest.

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