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Research Paper

Evaluation of medicinal potential and antibacterial activity of selected plants against *Streptococcus mutans*

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The aim of the study is to screen the bioactive compounds (saponin, tannin, phenolic compounds, terpenoid & steroid) present in selected ethnomedicinal plants, *Terminalia bellirica* (fruits), *Smilax zeylanica* (leaves) and *Dioscorea oppositifolia* (fruits) from Odisha state, India. The single formulation was prepared using the selected plants parts in the ratio 1 : 6 : 3 respectively for quantitative analysis of tannin & total phenol, antioxidant activity and analysis of MIC (Minimum Inhibitory Concentration) against *Streptococcus mutans* causing bacteria of tooth decay. Results revealed that selected plant parts are rich source of bioactive compounds like tannin, phenolic compounds and saponin. The quantitative analysis of secondary metabolites showed highest concentration of tannin. It was noted that antioxidant activity is highest in methanol extract as compared to aqueous and acetone. MIC analysis also revealed that formulated powder had excellent antibacterial activity against *S. mutans* and it was observed the lowest values (450 µg ml^{-1}) showed aqueous & methanol followed by acetone. The herbal formulation might be used to formulate new herbal products against tooth decay in near future.

Keywords: antibacterial activity, antioxidant activity, ethnomedicinal plants, secondary metabolites, tooth decay

1 Introduction

Tooth decay or dental caries or cavity causes the spoiling of tooth, is a complicated issue worldwide (Young et al., 2009). It is also associated with diabetes, high blood pressure and heart disease. *Streptococcus mutans* is the main cause of tooth decay. It has several virulence factors that are linked with cariogenicity. *Streptococcus mutans* adhere the sugar molecules near the tooth surface which result in the formation of sticky white, yellow and brown color patches called as plaque (Wong et al., 2013) which is a biofilm formed by the association of bacteria and the food in the oral cavity. The bacteria convert the sugar molecules to acid by the process of fermentation which results in tooth enamel erosion and soften the teeth tissues (Moore, 1983). Once the enamel is damaged by the acid, it affects the next layer biotin followed by damaging the pulp and root canal which causes serious pain and can be transmitted from mother to children during pregnancy too (Smith et al., 2002). Several other factors like poor dental hygiene, medication, dry mouth, less saliva, Sjögren´s syndrome, diabetes mellitus etc causes tooth decay (Neville and Day, 2002). There are lots of medications available against tooth decay like allopathic, homeopathic, herbal care and traditional therapeutic practices. In some cases, endodontic therapy is also useful (Nations and Nuto, 2002; Mijare and Mjor, 2003; Anderson, 2004; Giuca et al. 2010; Mast et al. 2013; Ferraz et al., 2012). Many bio-chemical metabolites & minerals help to prevent the teeth decay like fluoride can inhibit the effect of enolase as well as chlorhexidine which act against the bacterial adherence and help in remineralization of tooth enamel and decrease the effect of acid produced by bacteria (Kanduti et al., 2016) whereas polyphenols show the excellent inhibiting

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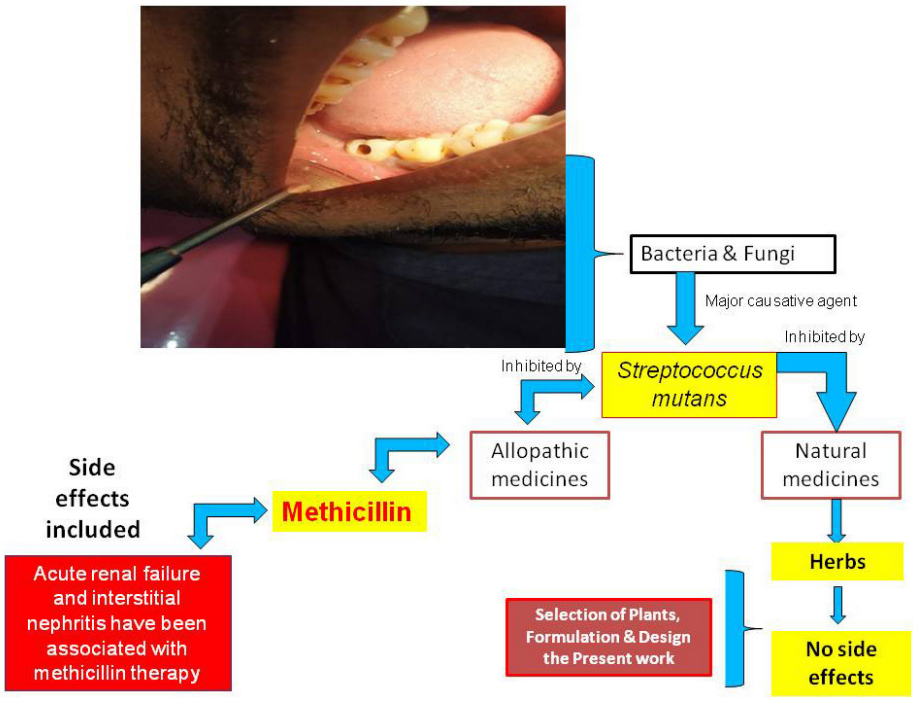

Figure 1 The demand and importance of herbal products against teeth decay

action against *Streptococcus mutans*, teeth problems (Ferrazzano et al., 2011). Other secondary metabolites could be effective against teeth problems like alkaloid, glycoside, terpenoid, aspirin, atropine, artemisinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol and tubocurarine (Gupta et al., 2015). The available synthetic medicines show good results on responsible pathogens for teeth decay but some has several adverse effects (Shekarchizadeh et al., 2013). The habit of using plants as medicine was seen from ancient time due to their better adaptability and compatibility with the human body and

Table 1 Plants used against toothache & other problems of teeth among the locals of Odisha state, India

Plant name	Family	Uses	Parts used
Acacia nilotica L.	Mimosaceae	swollen gum	bark
Acalypha indica L.	Euphorbiaceae	toothache	leaves
Achyranthes aspera L.	Amaranthaceae	toothache	leaves
Allium sativum L.	Liliaceae	toothache	bulbs
Aloe vera L.	Asphodelaceae	to clean the teeth	leaves
Anacardium occidentale L.	Anacardiaceae	toothache, sore & gum	whole plants
Argemone mexicana L.	Papaveraceae	toothache	seeds
Azadirachta indica A. Juss.	Meliaceae	toothache & sores	stem
Borassus flabellifer L.	Arecaceae	toothache	fruits
Calotropis gigantea L.	Asclepiadaceae	toothache	latex in very small amount
Cassia occidentalis L.	Caesalpiniaceae	toothache	leaves and seeds
Cinnamomum camphora L.	Lauraceae	toothache	leaves
Curcuma longa L.	Zingiberaceae	toothache & gingivitis	rhizomes
Justicia adhatoda L.	Acanthaceae	Pyorrhoea	leaves
Nicotiana tabacum L.	Solanaceae	toothache	leaves in small amount
Ocimum sanctum L.	Lamiaceae	mouth sores	leaves and stem

less side effects (Figure 1). For the treatment of tooth decay, there are several plants used to inhibit the growth of bacteria *Streptococcus mutans* (Sharma et al., 2018). Therefore, need to find an alternative medications have zero side effects from plant wealth.

Keeping this in view, the present study has designed on the traditional practices using plant parts and highlights the importance of plant wealth to screen new herbal agents against teeth problems. The survey works has done from different parts of country on plants used against teeth problems (Table 1) and three plants (*Terminalia bellirica*, *Smilax zeylanica* and *Dioscorea oppositifolia*) were selected for experimental works for scientific validation of tribal claims and providing a base line data for formulation of herbal drugs possess zero side effects against toothache problems.

2 Material and methods

2.1 Selection, identification and collection of selected experimental plants

Smilax zeylanica, *Dioscorea oppositifolia* and *Terminalia bellirica* were selected for experimental works. *Smilax zeylanica* leaves were collected from different area of Khordha district, Odisha. *Dioscorea oppositifolia* fruits were collected near Khandagiri hill (Khordha). *Terminalia bellirica* fruits were collected from the tribal area of Khordha, Odisha. The experimental plants were identified by corresponding author using Flora's book (Haines, 1922; Saxena and Brahmam, 1994).

2.2 Preparation of the plant powdered extracts

After collection of all the experimental plants, they were properly washed with water. Then they were allowed to dry under room temperature for about 2 to 3 days and after drying the parts were grounded separately with the help of mixer grinder and stored in different containers which were leveled for easy identification.

2.3 Phytochemical assay

Phytochemical analysis was carried out on different extracts of experimental plant parts by using Soxhelt apparatus. n-hexane, methanol, acetone, ethanol and aqueous were selected for extraction as per polarity index (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993; Raaman, 1993).

Test of saponin

About 5 ml of the filtrate was taken in a test tube and 2 ml of distilled water was added and shaken properly. The presence of saponin can be indicated by the presence of stable persistent or soapy foaming substances.

Test of Tannin

2 ml of filtrate solution was taken in a test tube and 3 to 5 drops of 0.1% ferric chloride was added. The brownish green or blue black coloration indicates the presence of tannin.

Test of Phenolic compounds

3–4 drops of 1% ferric chloride solution was added to 2 ml of filtrate, formation of bluish black coloration indicates the presence of phenolic compounds.

Test for Terpenoid

About 3 ml of filtrate was taken in a test tube. 3 to 4 drops of chloroform followed 4 to 5 drops of concentrated sulfuric acid was added. A reddish-brown coloration of interface indicates the presence of terpenoid.

Test for Steroid

About 3 ml of filtrate was taken in a test tube and 3 to 4 drops of chloroform & 4 to 5 drops of concentrated sulfuric acid is added to it separately. A reddish-brown coloration indicates the presence of steroid.

2.4 Preparation of formulation

30 g of *Smilax zeylanica* (leaves), 15 g of *Dioscorea oppositifolia* (fruits) and 5 g of *Terminalia bellirica* (fruit) were mixed to form a mixture or formulation in a percentage of 60%, 30%, 10% respectively. 10% of Terminallia bellirica fruits were taken. About 60% *Smilax zeylanica* leaves were taken for formulation. About 30% of *Dioscorea oppositifolia* fruits were added.

2.5 Quantitative test of phenol and tannin

Extraction of phenol

0.5 g of powdered sample of mixed formulation was taken in a beaker and 5 ml of 60% methanol is added. Then it is kept for about 30 min in room temperature. After that, the mixture was filtered and kept in another beaker (Deshmukh and Theng, 2018).

Estimation of phenol

6 dry clean test tubes were taken including a blank one and leveled. In the blank test tube, 1 ml of 60% methanol was added. Then about 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml of filtrate was added to the 1st, 2nd, 3rd, 4th, 5th test tubes respectively. Then the volume was made up to 1 ml each by adding required amount of 60% methanol i.e. 900 µl, 800 µl, 700 µl, 600 µl and 500 µl to 1st, 2nd, 3rd, 4th, 5th test tube separately. About 1 ml of HCl and 1ml of Sodium molybdate were added to each test tube including blank one, and the solutions were shaken properly. The solution was kept about 20 min at room temperature.

5 ml of distilled water was added to the solution and kept for about 20 min. After 20 min, 2 ml of NaOH solution was added to it and kept for 20 min and then the reading were taken in 515 nm using spectrophotometer (AIE Spectro) and recorded (Deshmukh and Theng, 2018).

Extraction of tannin

0.5 g of powdered sample was taken in a beaker and about 75 ml of distilled water was added to it. It was boiled for about 30 min. After boiling, it is allowed to cool in room temperature. Then it is centrifuged at 2000 rpm for 5 min. The supernatant was taken in a different beaker. About 1 ml of supernatant was mixed with 75 ml of distilled water. To it 5 ml of folin's reagent, 10 ml of sodium carbonate were added. Then the whole solution was shaken for about 5 min and after it, the reading were taken at 720 nm using spectrophotometer (AIE Spectro) and recorded (Deshmukh and Theng, 2018).

Estimation of tannin

0.5 mg of tannic acid was mixed with about 1 ml of distilled water and from this solution 5, 10, 15, 20, 25 µl were taken in different test tubes. The volume was made up to 1 ml by adding distilled water. 0.5 ml of folin's reagent and 2.5 ml of sodium carbonate were added to each test tube. The solutions were shaken properly in dark condition and left for about 40 min. After 40 min the readings were taken at 720 nm compared with standard Gallic acid (Deshmukh and Theng, 2018).

2.6 Antioxidant test

For antioxidant test, metal chelating scavenging activity was done.

Metal chelating scavenging activity

The metal chelating activity of plant extract was determined using (Gouda et al., 2013). This test was done in different extract like aqueous, methanol and acetone. About 5 g of the formulated sample was taken in 3 different beakers and about 50 ml of water, methanol and acetone were added to the beakers respectively. Then the beakers containing the solvent and sample were kept in fridge for about 24 h. After that the solution were filtered and leveled, 6 dry clean test tubes were taken including a blank one. 1 ml of distilled water added to the blank test tube. 10 mg ml^{-1}, 20 mg ml^{-1}, 30 mg ml^{-1}, 40 mg ml^{-1} and 50 mg ml^{-1} of the filtered sample were taken in 1st, 2nd, 3rd, 4th, 5th test tubes accordingly. About 0.5 ml of ferrous chloride solution was added to the test tubes and kept for 40 min in room temperature. After 40 min, 0.2 ml of ferozin added to all the test tubes and kept for 15 min. Then the readings were taken at 562 nm using spectrophotometer (AIE Spectro) and recorded. Butylated hydroxytoluene (BHT) was taken as standard solution calculation.

$$\% \text{ scavenging} = \frac{A_0 - A_1}{A_0} \times 100$$

where: A_0 – absorbancy of control; A_1 – absorbancy of test sample

2.7 Estimation of MIC (Minimum Inhibitory Concentration)

All the extracts of formulated powder were screened for their antibacterial activity. Antibacterial activity was assessed by MIC by serial dilution method. The used MTCC 497 (Microbial Type Culture Collection) bacterial strain (*Streptococcus mutans*) collected from Institute of Microbial Technology (IMTECH), Chandigarh. Selected colonies of aforesaid bacteria were picked off to a fresh isolation plate and inoculated in corresponding tubes containing 5 ml of trypticase soy broth. The broth was incubated for 8 ±1 h at 35 ±2 °C until there was visible growth. Mc Farland No. 5 standard and PBS (Phosphate buffer saline) were used to adjust the turbidity to get 10^5 CFU ml^{-1} (Rai et al., 2010). Kanamycin is used as standard antibiotic.

3 Results and discussion

The present study emphasizes on the phytochemical test indicating the presence or absence of bioactive compounds through qualitative test. Phytochemical test of *Smilax zeylanica* (leaves), *Terminalia bellirica* (fruit) and *Dioscorea oppositifolia* (fruit) was carried out with different extractants like aqueous, methanol, ethanol, acetone and n-hexane (Table 2). It was observed that in leaves of *Smilax zeylanica*, phenolic compound present in all extracts except ethanol and n-hexane. Phenolic compound is more abundant in methanolic as well as acetone extract. Terpenoid and steroid are absent in methanolic, ethanolic and n-hexane while present in aqueous and acetone. Tannins are comparatively more in aqueous extract than acetone and ethanol extractant but absent in others whereas Dhanya et al. (2018) reported that leaves showed the presence of alkaloids, flavonoids, saponins, tannin, glycosides, triperpenoids and phenolic compounds. *Dioscorea oppositifolia* (fruits) showed absence of almost all tested bioactive compounds like saponin, tannin, phenolic compound, terpenoid and steroid in acetone, ethanol and n-hexane extractants. Saponin is the found in methanolic extract. In aqueous, saponin, phenolic compounds, tannin, terpenoid and steroid are detected (Table 3). The literature survey revealed that no or less reports are available on the bioactive compounds present in the fruits of *Dioscorea*

oppositifolia. It was also observed that *Terminalia bellirica* fruits showed the presence of phenolic compounds in almost all extracts except n-hexane. Tannin is found in aqueous followed by methanol, ethanol, acetone but absence in n-hexane. Terpenoid and steroid are not detected while Saponin is only detected in aqueous extract (Table 4) whereas Hazra (2019) documented the bioactive compounds present in the fruits of *Terminalia bellirica* (Alkaloid, flavonoid, glycoside, terpenoid, tannin). After the phytochemical analysis of selected plants, single formulation was carried out using the experimental plant parts for quantitative analysis of secondary metabolites, antioxidant activity and antibacterial activity. After the quantification of tannin and total phenol, It was observed that tannin showed highest concentration followed by total phenol (Figure 2). It was examined that aqueous extract showed highest scavenging activity followed by methanol & acetone (Figure 3). It showed the sound antioxidant activity of formulated herbal powder of experimental plant parts. Many researchers have reported the total phenol, tannin and antioxidant

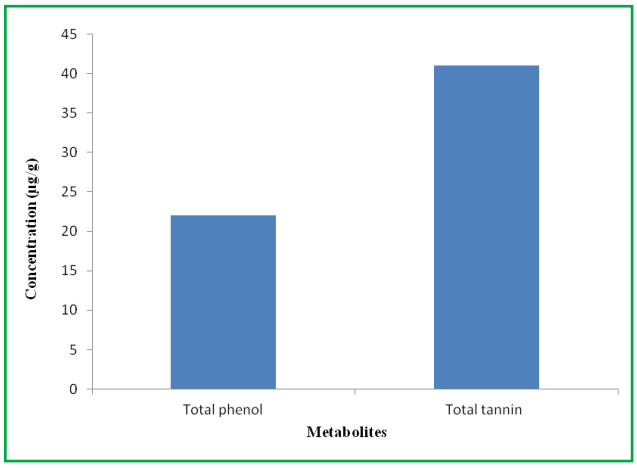

Figure 2 Bar chart representing concentration of secondary metabolites

Table 2 Phytochemical assay of *Smilax zeylanica* leaves

Bioactive compound	Aqueous	Methanol	Acetone	Ethanol	n-hexane
Saponin	+	-ve	+	-ve	-ve
Tannin	+++	-ve	++	++	-ve
Phenolic compound	+	+++	+++	-ve	-ve
Terpenoid	+	-ve	+	-ve	-ve
Steroid	+	-ve	+	-ve	-ve

+ low; ++ mild; +++ high

Table 3 Phytochemical assay of *Dioscorea oppositifolia* fruits

Bioactive compound	Aqueous	Methanol	Acetone	Ethanol	n-hexane
Saponin	+++	++	-ve	-ve	-ve
Tannin	+	-ve	-ve	-ve	-ve
Phenolic compound	+++	-ve	-ve	-ve	-ve
Terpenoid	+	-ve	-ve	-ve	-ve
Steroid	+	-ve	-ve	-ve	-ve

+ low; ++ mild; +++ high

Table 4 Phytochemical assay of *Terminalia bellirica* fruits

Secondary metabolites	Aqueous	Methanol	Acetone	Ethanol	n-hexane
Saponin	+	-ve	-ve	-ve	-ve
Tannin	+++	++	++	++	-ve
Phenolic compound	+++	+++	+++	+++	-ve
Terpenoid	-ve	-ve	-ve	-ve	-ve
Steroid	-ve	-ve	-ve	-ve	-ve

+ low; ++ mild; +++ high

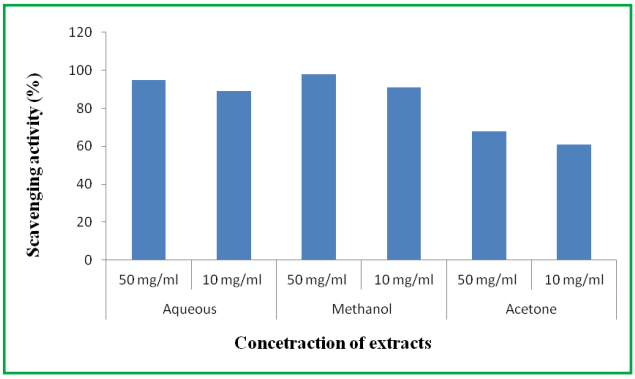

Figure 3 Bar chart depicting antioxidant activities of formulated sample

activities of selected plant parts (Jyothi et al., 2012; Hazra, 2019) but nor or les reports on formulation.

The estimation of MIC shoed the sound antibacterial activity of formulated herbal extract. It was observed that aqueous & methanol extract showed MIC at 450 µg ml^{-1} whereas acetone extract showed 500 µg ml^{-1} (Table 5) as compared to Kanamycin (12.5 µg ml^{-1}) and Ampicillin (3.125 µg ml^{-1}). There is no or less reports are available on antibacterial activity of formulated herbal against *S. mutans* which show its novelity.

Table 5 Estimation of MIC of formulated powder against *S. mutans*

Sample(s)	Concentration
Acetone	500 µg ml^{-1}
Methanol	450 µg ml^{-1}
Aqueous	450 µg ml^{-1}
Ampicillin	3.125 µg ml^{-1}
Kanamycin	12.5 µg ml^{-1}
Control	growth in all concentration
Broth control	no growth

4 Conclusion

Teeth decay is a major and common problems throughout the world which increase as per the age. There are number of synthetic drugs are available to treat the problems related to teeth but people want to use herbal drugs. The whole world is declining towards organic medications due to less or no side effects. Hence, to reduce the negative impacts of synthetic drugs used in problems related to teeth and as per the increasing interest on herbal products, present study has designed to validate scientifically the ethnomedicinal practices against teeth problems. The present works conclude that selected plant parts have diverse bioactive compounds which may be responsible to inhibit or kill the pathogens responsible for teeth decay such as saponin, tannin and phenolic compounds. The antioxidant and antibacterial potential of formulation of experimental plant parts show the sound medicinal potential. The methanol extract of formulated powder showed highest antibacterial and antioxidant activities. In future, it may be used to formulate herbal drugs against teeth problems.

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