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Validation of Tribal Claims on *Cucumis melo* L.

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ABSTRACT

Rural and tribal populace use different types of plants against tooth ache having less or no side effects. Keeping this in view, an attempt was made to gather the information on plants used in tooth problems by the local community of Similipal Biosphere Reserve (SBR) and its peripheral areas. Validation of the claims was done using phytochemistry and antibacterial analysis. The survey revealed that the local people used *Cucumis melo* fruits as food against tooth decay and microbial infections. The phytochemical analysis and antimicrobial activities against *Streptococcus mutans* (MTCC 497) were analyzed and found that the *C. melo* fruits contained diverse bioactive compounds and showed antibacterial activity against tested pathogenic bacteria. The results validated tribal claims. The present study highlights the importance of wild nutraceutical in the formulation of future drugs against tooth decay and microbial infection.

Key words: *Cucumis melo*, Similipal Biosphere Reserve, *Streptococcus mutans*, tooth ache, traditional therapeutic systems

INTRODUCTION

Diseases related to tooth are very common throughout the world. It is an important healthcare issue as per WHO (World Health Organization). Dental caries, periodontal diseases, trauma, occlusal dysfunction and aches are common tooth problems. The diseases occur due to unhygienic conditions and microbial infections (Nazir, 2017). About 750 species of bacteria inhabit the oral cavity and a number of these are associated with infections. The most responsible bacteria are acidogenic and aciduric (Sharma *et al.*, 2018). They are *Streptococcus, Lactobacillus* and *Actinomycetes* species which are responsible to dissolve the calcium phosphate from tooth. There are a number of allopathic medicines available against tooth problems, most of them having some side effects (Sahoo *et al.*, 2021). Therefore, the whole world is searching the herbal parts or products having fewer or no side effects. Keeping the importance of herbal parts/products and global tooth problems, an attempt has been made to screen a plant used in traditional therapeutic practices by the local communities of Similipal Biosphere Reserve periphery, Odisha, India.

SBR is located in the central part of the district, close to the interstate boundary with West Bengal in the North-East direction and Jharkhand in the North-West. It contains three protected habitats within its precincts, namely, Simlipal Tiger Reserve, Sanctuary, and National Park. The SBR is having a compact mass of natural forests spread over an area of 5,569 sq. km lying between 21° 10' to 22° 12' N and 85° 58' to 86° 42' E, ranging between 300 to 1180 m above sea level with numerous rolling hills with semi-evergreen forest, moist deciduous forest, dry deciduous hill forest, Sal forest, grassland and savanna.

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The dominant tribes in the district are Kharia, Mankdias, Santhal, Kol, Bhumija, Bhuyan, Mahalis, Sounti and Saharas. Some of the tribes, namely, Kharia and Mankdias still follow the primitive way of living. These tribes even today depend upon the wild foods available in the forest zones.The ethnic tribal communities of SBR are habitually forest lovers and commonly depend upon plants for their food and medicine (Kumar *et al.*, 2017). The tribal communities are knowledgeable in utilizing traditional ethnobotanical knowledge, using plants and their parts in curing diseases. The knowledge flows from generation to generation which has emerged as traditional medicines being practiced by local tribal priests. They collect and domesticate medicinal plant species which are having therapeutic values to cure different diseases. The plant parts are medicinally used in the form of paste, juice, powder ash, etc. (Kumar, 2015) in the crude form as per the requirement during treatment. Information during the field survey revealed that the aboriginals normally utilized herbal plant parts to cure tooth problems like the stem of neem (*Azadirachta indica*) and karanja (*Millettia pinnata*), fruit of bhuinkakharu (*Cucumis melo*), etc. Among them, they frequently use the fruit parts of *Cucumis melo*, a herbal climber. It belongs to the family Cucurbitaceae. It is a procumbent annual herb, leaves orbicular or ovate with shallow rounded and mucronate nerve ending; calyx tube campanulate, pubescent corolla; fruits spherical or ovate-obtuse at both ends. It is frequently available near forest fringes and paddy field near study areas. The present study highlighted the importance of wild nutraceutical in curing dental problems as well as validation of claims for fast future formulation against microbial infections and antimicrobial resistance.

MATERIALS AND METHODS

A field survey was carried out from 2018 to 2020 in different seasons in peripheral areas of SBR, Odisha, India. The information on *Cucumis melo* used as food and as traditional medicine against different diseases and disorders were collected through semi-structured questionnaires in the form of PDF (Passport Data Form) with different tribal communities of SBR. The claims were confirmed by cross checking them with informants. The identification of the plant was done following available literature (Kumar, 2015).

The plant materials for detection of bioactive compounds and evaluation of antibacterial activity were collected from Padampur village of SBR periphery. Collected plant materials were washed thoroughly by tap water followed twice by distilled water and were air-dried. The dried materials were crushed to powder with a mechanical device and were kept in an airtight container for phytochemical and antibacterial analysis.

The plant extract was prepared using the percolation method from 5 g of leaf powder which was macerated in a solvent (50 ml) for 12 h in the refrigerator. After 12 h the sample was filtered and the residue was again macerated in the same solvent. For each solvent, process was repeated thrice. The aqueous extract was prepared separately by taking the powder in distilled water followed by filtration. The filtrate was dried and concentrated to get a semisolid mass.

Phytochemical screenings were done using the standard methods. 0.7 ml of the extract was dissolved in 50 ml distilled water and was heated for 10 min. After cooling, few drops of 1% ferric chloride were added. The colour of the sample changed from yellow to green and a dark green precipitate was formed indicating the presence of tannins. Five ml of extract was dried and to it 1 ml of ethyl acetate was added. Ethyl acetate was removed and 2 ml of distilled water was added and the mixture was shaken vigorously. There was persistent foam formation which lasted for at least 15 min indicating the presence of saponins.

Two ml of leaf extract was taken in a flask and dissolved in two or three drops of 10% NaOH. A deep yellow colour developed which gradually became colourless by adding a few drops of HCl. It indicated the presence of flavonoids. One ml of the extract was mixed with 0.5 ml of chloroform. To this mixture equal volume of concentrated sulphuric acid was added. A reddish-brown interface indicated terpenoid presence. Five ml of extract was stirred with 3 ml of 1% aqueous HCl on the water bath and then filtered. One ml Dragendorff's reagent was added to the filterate. The occurrence of orange-red precipitate indicated the presence of alkaloids in the sample extract. Two ml of extract was treated with 3-4 drops of ferric

chloride solution. The formation of bluish-black colour indicated the presence of phenolic compounds. Five ml of extract was mixed with 2 ml of chloroform. The solution was cooled well in ice followed by the addition of concentrated H_2SO_4 carefully. In the lower chloroform layer red colour appeared that indicated the presence of steroids.

S. mutans is a major agent in dental caries vis-à-vis its capacity to orchestrate changes in the plaque microbiome via EPS and acid production (Lemos *et al.*, 2019). The extracts of *C. melo* fruits were screened for antibacterial activity against Gram-positive bacteria *Streptococcus mutans* (MTCC 497). The used MTCC (Microbial Type Culture Collection) bacterial strain was collected from the Institute of Microbial Technology (IMTECH), Chandigarh. Antibacterial activity was done using Agar Well Diffusion assay and Disc diffusion assay (Sahoo *et al.,* 2021) and broth Dilution assay (Nayak *et al.*, 2015). Kanamycin served as standard drug control. Triplicates were maintained and the experiment was repeated thrice. For each replicate the readings (diameter of zone of inhibition in mm) was taken and the mean ± SD values (diameter of zone of inhibition) were recorded.

Stock culture of MTCC 497 was maintained at 4°C on slants of semi-solid media containing 1.5% agar, 0.3% beef extract and 0.5% peptone. Active working cultures for experiments were prepared by transferring a loopful of culture mass from the stock. Slants were incubated for 24 h at 36±1.0°C.

Colonies of prepared slants of MTCC 497 were picked off using a sterile loop and inoculated in sterile conditions in an autoclaved cool liquid broth medium containing 0.3% beef extract and 0.5% peptone. The broth was incubated for 12 h at 36±1.0 °C until there was visible growth indicated by turbidity standard. Mean and SD (standard deviation) were calculated to evaluate triplicate values of a zone of inhibition (mm) of samples using Excel, Microsoft Corporation-2010, US.

RESULTS AND DISCUSSION

The survey work of the present study revealed that the community of SBR consumed the fruits of *C. melo* as food. It was noticed that they used fruits of *C. melo* after successive boiling (Table 1) may be due to the presence of anti nutritional factors. The ethnobotanical survey showed that the fruits were used in kidney problems, jaundice, mouth odour, tooth problems and stomach pain (Table 2; Fig. 1). It was also observed that they collected the mature fruits from near forest and paddy fields and burnt them. The ash was used to clean the tooth and paste made by water was applied to the gums. The ash boiled with water was used as mouth wash to remove the unpleasant mouth odour (Table 2).

Keeping the claims in view, the screening of bioactive compounds was done in four extracts: n-butanol, acetone, methanol and aqueous. It was observed that it contained tannins, phenolic compounds and saponins as major secondary metabolites (Table 3). The antibacterial activities were carried out against *S. mutans* using AWD (Agar Well Diffusion), DD (Disc Diffusion) and BDA (Broth Dilution Assay). It was noted that the fruit extracts were able to inhibit the growth of *S. mutans*. In, AWD assay, it was observed that the methanol and aqueous extract showed the highest zone of inhibition (ZI) against *S. mutans* at 0.5 mg/ml concentration (Table 4). In DD assay, methanol and aqueous extracts showed the highest ZI followed by acetone and

Table 1. Traditional food systems of *C. melo* (fruits) among the aboriginals of SBR

Collection site (s)	Informant(s)	Part(s) used	Palatability	Mode of consumption and culinary use(s)
Padampur	Ramu Ho	Fruits	Moderate	The young fruits are collected and boiled. Water is removed. The boiled fruits are fried and taken with the main meal.
Jashipur	Balram Munda	Fruits	Rare	The young fruits are collected and boiled. After successive boiling, fruits are taken out and dried. The dried fruits are cooked with tamarind fruits and chilli.
Angarpada	Upendra Nayak	Fruits	Rare	The fruits are collected and seeds are removed. The main mesocarp of the fruits is then boiled to remove the bitterness. Fruits are then fried and taken with rice.

Table 2. Ethnobotanical values of *C. melo* among the aboriginals of SBR

Plant parts	Used against	Mode of uses
Fruits	Kidney stone (pathri)	2-3 young fruits are taken and paste is prepared with water. The paste is then mixed with a glass of date palm juice on empty stomach in the early morning.
Fruits	Jaundice	Young female flowers with immature young fruits are crushed with date palm stem juice (collected early in the morning). One glass of juice is taken once in the early morning on empty stomach till cure.
Fruits	Stomach pain	2-3 pericarp of young fruits are taken and eaten raw to reduce stomach pain.
Fruits	Mouth odour	Mature fruits are dried and powdered. The powder is put into a local cigarette (bidi) made up of leaves of *Diospyros melonoxylon* and smoked to cure mouth odour.
Fruit	Tooth problems	Mature fruits are collected from near forest, paddy fields and are burnt . The ash is used to clean the tooth as paste made by water is applied on gum against tooth decay and toothache.

Fig. 1. Collection of ethnomedicinal information and plant parts of *C. melo*.

Table 3. Phytochemical screening of *C. melo* (fruits)

Plant parts	Solvents used	Detected bioactive compounds
Fruits	n-butanol	Not detected
	Acetone	Phenolic compounds and flavonoids
	Methanol	Reducing sugars and Steroids
	Water	Tannins, flavonoids, phenolic compounds, saponins and reducing sugars

Table 4. Antibacterial activity of *C. melo* fruit using AWD assay against *S. mutans*

Extracts		Zone of inhibition
0.25 mg/ml	n-Butanol	No inhibition
0.5 mg/ml	n-Butanol	No inhibition
0.25 mg/ml	Acetone	7.00±0.00
0.5 mg/ml	Acetone	8.00±0.00
0.25 mg/ml	Methanol	10.00±0.00
0.5 mg/ml	Methanol	11.00±0.00
0.25 mg/ml	Aqueous	9.00±0.00
0.5 mg/ml	Aqueous	10.00±0.00

Table 5. Antibacterial activity of *C. melo*

Extracts	Zone of inhibition	Concentration
n-Butanol	No inhibition	10 µg/disc
Acetone	ZI ? 7.00	
Methanol	ZI ? 7.00	
Aqueous	8.00±0.00	
n-Butanol	No inhibition	20 µg/disc
Acetone	8.00±0.00	
Methanol	10.00±0.00	
Aqueous	10.00±0.00	
n-Butanol	No inhibition	30 µg/disc
Acetone	8.00±0.00	
Methanol	11.00±0.00	
Aqueous	11.00±0.00	

n-butanol (Table 5). The AWD and DD assay showed its antibacterial potential. Therefore, the MIC (Minimum Inhibitory Concentration) was performed and it was noted that the MIC was observed at 300 µg/ml with methanol and aqueous extract followed by acetone at 500 µg/ml (Table 6).

Several studies have been carried out on ethnomedicinal plants but very few studies have been reported on validation of tribal claims through phytochemistry and antibacterial activites. The food values of *C. melo* were reported by researchers (Kumar, 2015; Tripathy *et al.,* 2015) but the present study highlights its traditional food systems and how to remove the anti-nutritional factors

Table 6. MIC of *C. melo* fruits against *S. mutans*

Plant extracts	Concentration					
	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	1000 µg/ml
n-butanol	Growth	Growth	Growth	Growth	Growth	Growth
Acetone	No growth	No growth	No growth	No growth	No growth	Growth
Methanol	No growth	No growth	No growth	Growth	Growth	Growth
Aqueous	No growth	No growth	No growth	Growth	Growth	Growth
Inoculum control	Growth	Growth	Growth	Growth	Growth	Growth
Broth control	No growth	No Growth	No Growth	No Growth	No Growth	No Growth
Positive control Kanamycin (12.5 µg/ ml)	No growth	No growth	No growth	No growth	No growth	No growth

using successive boiling. The phytochemical screening of *C. melo* fruits indicated the presence of tannins and phenolic compounds. Tannins and phenolic compounds may be responsible to cure tooth decay and microbial infections (Kharouf *et al.*, 2020; Sudhakar and Raman, 2020). Recently, Sahoo *et al.* (2021) reported that tannins present in plant parts might be responsible to cure tooth decay and tooth ache problems.

To reduce the side effects of allopathic medicines and for sustainability of nature and health care, increased awarenress of the herbal products is very important. The present study brings attention towards green therapy. The validation of tribal claims on the selected plant, *C. melo* in the present study highlights its medicinal potential with no or fewer side effects. The phytochemical screening and antibacterial activity of *C. melo* fruit shows its potential to act against the infections caused by *S. mutans*. This encourages formulation of new herbal drugs to overcome antimicrobial resistance with no side effects.

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