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Antimicrobial activity of *Drosera peltata* J.E.Sm extracts against clinically isolated human cariogenic pathogens—an *in vitro* study

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Abstract: Oral cariogenic bacterial infection causes development of dental caries. This caries become intensely painful and hard to eradicate these causing agents without side effect. The plant and the phytoconstituents have a promising therapeutic potential in the management of dental caries treatment. The study aimed to evaluate antimicrobial activity of chloroform, ethanol and aqueous extracts of *Drosera peltata* J.E.Sm against clinically isolated human cariogenic pathogens. Chloroform, ethanol and aqueous extracts of *D. peltata* were tested against the *Streptococcus mutans* (a bacterium) and *Candida albicans* (a non-filamentous fungus). They are causing dental caries and were isolated from caries infected patients. The above three extracts showed antimicrobial activity against both *S. mutans* and *C. albicans*. The zone of inhibition was found to be high with chloroform extract of *D. peltata*. It also showed the minimum inhibitory concentration against *S. mutans* than the aqueous and ethanol extracts to Mueller Hinton Broth (MHB).The plant *D. peltata* showed antimicrobial activity against dental caries such as *S. mutans* and *C. albicans*. It would be have therapeutic use for dental caries and other oral infections.

Keywords: *Drosera peltata*, *Streptococcus mutans*, *Candida albicans*, antimicrobial, zone of inhibition.

Drosera peltata J.E. m'nin klinik olarak izole edilmiş insan karyojenik patojenlerine karşı ekstraktlarının antimikrobiyal aktivitesi - bir in vitro çalışma

Özet: Oral karyojenik bakteriyel enfeksiyon diş çürüğünün gelişmesine neden olur. Bu çürükler şiddetli ağrılı hale gelir ve bu neden olan ajanların yan etkisi olmadan ortadan kaldırılması zorlaşır. Bitki ve fito-bileşenlerin diş çürüğü tedavisinin tedavisinde umut verici bir terapötik potansiyeli vardır. Çalışma, klinik olarak izole edilmiş insan karyojenik patojenlerine karşı *Drosera peltata*'nin kloroform, etanol ve sulu ekstraktlarının antimikrobiyal aktivitesini değerlendirmeyi amaçladı. Kloroform, etanol ve *D. peltata*'nin sulu özleri, *Streptococcus mutans* (bir bakteri) ve *Candida albicans* (filamentöz olmayan bir mantar) karşı test edildi. Diş çürüğüne neden olurlar ve çürük bulaşmış hastalardan izole edilirler. Yukarıdaki üç ekstrakt hem *S. mutans* hem de *C. albicans*'a karşı antimikrobiyal aktivite gösterdi. İnhibisyon bölgesinin, *D. peltata* kloroform özütü ile yüksek olduğu bulunmuştur. Aynı zamanda, *S. mutans*'a karşı, Mueller Hinton Broth'a (MHB) sulu ve etanol özütlerinden daha düşük önleyici konsantrasyon gösterdi. mutans ve *C. albicans*. Diş çürüğü ve diğer oral enfeksiyonlar için terapötik kullanım olacaktır.

Anahtar Kelimeler: *Drosera peltata, Streptococcus mutans, Candida albicans*, antimikrobiyal, inhibisyon bölgesi.

1. Introduction

Dental caries is one of the infectious microbial diseases caused by the bacterium *Streptococcus mutans* which leads to localized dissolution and destruction of the calcified tissues of the teeth. In presence of fermentable carbohydrates, this bacterium causes damage by liquefying tooth structures in result in dental caries. This dental caries and periodontal diseases are the most predominant and preventable infectious diseases in this world. The availability of antibiotics, associated adverse effects and cost of medicine used in dentistry, made dentists to consider natural medicines for dental treatment. In recent years, the phytoconstiuents which are isolated from the plant sources were given more attention by researchers to find out a good alternative for various diseases of oral cavity (Shyla, 2011).

One among such an alternative is *Drosera* species. They contain physiologically and pharmacologically active compounds such as flavonoids and naphthoquinones such as plumbagin. Various scientific studies reported that these flavonoids and naphthoquinones possess various therapeutic effects such as anticancer, antifertility, anticonvulsant, anti-inflammatory and antimicrobial

moreover the aerial parts of *D. peltata* was reported to have antimicrobial activity of on oral bacteria which was evaluated by using agar diffusion and dilution micro methods (Raju, 2013a; Raju, 2013b). Researchers reported that ethanol and aqueous extracts of Indian *D. peltata* showed protection over the cancer associated metabolic syndrome developed in Dalton's ascites lymphoma (DAL) and Ehrlich's Ascites Carcinoma (EAC) bearing mice (Raju, 2016a). Similarly, its antibacterial effect was also reported against clinically isolated human periodontal pathogens such as *Prevotella intermedia, Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* (Raju, 2016b).

None of them investigated the antimicrobial effect of Indian *Drosera peltata* against dental caries caused by *S. mutans* (a bacterium) and *Candida albicans* (a non filamentous fungus). So the aim of present study was to investigate antimicrobial activity of chloroform, ethanol and aqueous extracts of *Drosera peltata* against clinically isolated human cariogenic pathogens.

2. Materials and methods

2.1 Plant materials

The whole plant of *D. peltata* was collected from Munnar hills, Kerala, India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. The material was washed, shade dried, powdered and stored in air tight containers for further experiments.

2.2 Preparation of the extracts

2.2.1 Chloroform extract:

A weighed quantity of the air-dried powdered plant drug was extracted with chloroform (60-80^{o}C) in a soxhlet apparatus.

2.2.2 Alcohol extract:

A weighed quantity of the air-dried powdered drug was extracted with ethanol (90 %v/v) in a soxhlet apparatus.

Both the extracts were concentrated in a rotary flash evaporator at a temperature not exceeding 50ºC.

2.2.3 Aqueous extract:

The marc from the ethanol extract was macerated with chloroform- water for 24h to obtain the aqueous extract. This was concentrated under reduced and dissolved in distilled water for experimental studies.

The chloroform (CEDP), ethanol (EEDP) and aqueous (AEDP) extracts of *D. peltata* were stored in air tight containers for experimental study.

2.3 Microorganisms:

S. mutans and *C. albicans* were isolated from the dental caries of infected patients from the Dental clinic, Marthandam, Tamil Nadu, India.

2.4 Media Used: Thioglycolate broth (TGB) and brain heart infusion broth (BHI) are the transport media used to maintain clinical dental caries sample in viable condition. Thioglycolate broth (TGB) contained per liter of deionised water: 15 g casein enzyme hydrolysate, 5 g yeast extract, 5.5 g dextrose, 2.5 g sodium chloride, L-cystine 0.5 g, 0.5 g sodium thioglycolate with a pH of 7.1 at 25°C. Brain heart infusion broth (BHI) contained, per liter of deionised water: 200 g calf brain infusion from, 250 g brain heart infusion from, 10 g protease peptone, 2 g dextrose, 5 g sodium chloride, 2.5 g disodium phosphate with a pH of 7.4 at 25°C.

Growth media used in examining the samples at aerobic condition includes, nutrient agar (NA), blood agar (BA) and MacConkey agar (MAC). Nutrient agar (NA) contained, per liter of deionised water: 5 g Hi veg peptone, 1.5 g Hi veg extract, 1.5 g yeast extract, 5 g sodium chloride, agar 15 g with a pH of 7.4 at 25°C. Blood agar (BA) is prepared by adding 20 mL of sheep blood to 200 mL of nutrient agar media as prepared like the above-mentioned composition. MacConkey agar (MAC) contained, per liter of deionised water: 17 g peptic digest of animal tissue, 3 g Protease peptone, 10 g lactose, 1.5 g Bile salts, 5 g sodium chloride, 0.03 g neutral red and agar 15 g with a pH of 7.1 at 25°C. For the examination of pathogenic fungi from dental caries sample, Sabouraud's dextrose agar (SDA) contained per liter of deionised water: 40 g dextrose, 10 g peptone and 20 g agar with a pH of 5.7 before autoclaving.

Growth media used to examine the samples at microaerophilic condition are brain heart infusion blood agar + 20% sucrose (BHIBA + 20% sucrose), thioglycolate agar (TGA) and trypticase yeast extract cystine sucrose bacitracin agar (TYCS20B) a medium for the selective isolation of *S. mutans* contained per liter of deionised water: 40 g trypticase soy agar (TSA), 5 g Bacto agar (Difco), 10 g yeast extract, 200 g sucrose. The medium was sterilized and cooled to 55°C 200 IE bacitracin was incorporated. BHI broth with agar served as brain heart infusion agar used for culturing of *S. mutans* under microaerophilic condition. TGB with agar 2 g served as thioglycolate agar (TGA) used for culturing of *S. mutans* under microaerophilic condition.

2.5 Sample collection: This procedure was carried out under aseptic condition, by using an excavator dental caries. Samples were collected and hosted into the 2mL TGB or BHI broth and later it was mixed well by using magnetic stirrer. After that the organisms were inoculated in their culture medium by streak plate technique.

2.6 Antimicrobial susceptibility assay

2.6.1 Disc diffusion assay

In this method, plates were prepared with 20mL of sterile BHI for *S. mutans* and MHA for *C. albicans*. The test cultures (100 µL of suspension containing 10^{8}CFU/mL bacteria (or microorganisms under study) were swabbed on the top of the solidified media and allowed to dry for 10min. The tests were conducted at three different concentrations of the crude extract (200 mg, 5mg and 2.5mg per disc) dissolved in 5% dimethyl sulfoxide (DMSO). The sterile 6mm disc (Himedia) impregnated with different concentrations of extracts. The loaded discs were placed on the surface of the medium and left for 30min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Penicillin and Amphotericin-B (5 and 10 µg /disc) were used as positive

control. The plates were incubated for 24 h at 37°C. Zone of inhibition was recorded in millimeters and the experiment was repeated twice (Paper, 2005; Sunaina 2013).

2.6.2 Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was performed according to the standard reference method (Paper 2005; Sunaina 2013).. The extracts were dissolved in water and 2% dimethyl sulfoxide (DMSO). The concentrations of extracts were prepared in 600, 300, 150, 75, 37.5, 18.75, 9.4, 4.7, 2.35 and 1.2 µg/ml respectively. The initial test concentration was serially diluted twofold. Each well was inoculated with 5 µL of suspension containing 10^8CFU/mL of bacteria and fungi. The antibacterial agent penicillin and antifungal agent Amphotericin-B were included in the assays as positive controls. The plates with bacteria were incubated for 24 h at 37°C.After incubation, 5 µL of tested broth was placed on the sterile MHA and BHI plates and incubated at respective temperature. The MIC for bacteria was determined as the lowest concentration of the extracts inhibiting the visual growth of the test cultures on the agar plate. Three replications were maintained.

2.7 Statistical analysis

All triplicate data were presented in mean ± Standard Deviation (S.D) manner.

3. Results

Table 1 showed the effect of CEDP, EEDP and AEDP on *S. mutans* and *C. albicans* isolated from the dental caries of infected patients by disc diffusion method. Three extracts were tested against *S. mutans*, and *C. albicans* where the zone of inhibition was measured and presented in millimetre. The results were compared with standard antibiotics such as Penicillin and Amphotericin B. The zone of inhibition was increased in a concentration dependent manner for all the extracts. The highest diameter of zone of inhibition (44.7±1.0) was found with 16mcg/mL of CEDP against *S. mutans* whereas 10 mcg/mL of Penicillin showed 47.6±3.1 and 44.3±1.2 for Amphotericin B. The zone of inhibition was 42.7±1.8 for *C. albicans* shown by CEDP. Similarly, the highest concentration of EEDP showed 34.8±3.6 and 33±1.6 for zone of inhibition against *S. mutans*, and *C. albicans* respectively. The result was comparatively like that of 5mcg/mL of standard Amphotericin B drugs. The highest dose of AEDP showed the maximum zone of inhibition (18±2.5) against *C. albicans*, however the results given by the AEDP was comparatively lesser than the standard and other extracts.

CEDP- chloroform extracts of *D. peltata*, EEDP – ethanol extracts of *D. peltata*, AEDP- aqueous extracts of *D. peltata*

Table 2 showed the MIC by visual observation produced by the extracts CEDP, EEDP and AEDP against *S. mutans* and *C. albicans*. The results were compared with standard antibiotics such as Penicillinand Amphotericin B. In a series of dilution, the MIC of CEDP was found to be least (2.35mcg/mL) than other extracts on *C. albicans* whereas it was the next higher dose (4.7mcg/mL) for *S. mutans*. Similarly, 1.25mcg/mL of Penicillin and 5 mcg/mL of Amphotericin B were showed inhibition of growth on all the tested pathogens. In case of EEDP the minimum concentration required for the inhibition of dental pathogens growth were 9.4 and 4.7mcg/mL against *S. mutans*and *C. albicans* respectively. AEDP consumed 18.75mcg/mL for growth inhibition of *C. albicans.*

Table 1 Zone of inhibition of extracts against *S. mutans* and *C. albicans* isolated from cariogenic pathogens.

Name of antimicrobial	Concentration per disc (mcg/mL)	Zone of Inhibition (mm)	
		Name of microorganism	
		S. mutans	*C. albicans*
CEDP	1	11.3±0.1	12.4±1.7
	2	18.6±2.3	20.3±0.5
	4	24.2±1.0	30.4±0.5
	8	36.1±2.1	36.1±3.2
	16	44.7±1.0	42.7±1.8
EEDP	1	7.2±0.01	5.8±1.7
	2	11.5±1.6	12.2±1.7
	4	21.5±3.4	21.9±0.8
	8	26.5±2.1	17.3±1.5
	16	34.8±3.6	33±1.6
AEDP	1	2.2±1.1	2.8±0.6
	2	9.6±1.3	7.5±1.1
	4	10.3±1.5	15.5±1.3
	8	11.3±0.9	16±1.8
	16	12.1±1.3	18±2.5
Penicillin	5	28.1±2.1	22.8±1.9
	10	47.6±3.1	39.4±1.1
Amphotericin B	5	31.7±3.2	38.1±2.6
	10	44.3±1.2	47±1.8

Table 2 Minimum Inhibitory Concentration (MIC) of CEDP, EEDP and AEDP by visual observation

Name of Antimicrobial	Minimum Inhibitory Concentration (MIC in mcg/mL)	
	Name of microorganism	
	S. mutans	*C. albicans*
CEDP	4.7	2.35
EEDP	9.4	4.7
AEDP	9.4	18.75
Penicillin	2.5	1.25
Amphotericin B	5	5

CEDP- chloroform extracts of *D. peltata*, EEDP- ethanol extracts of *D. peltata*, AEDP- aqueous extracts of *D. peltata*

4. Discussion

S. mutans is an acid producing pathogen which causes dental plaque and dental caries. It is characterized by bad breath and foul tastes. If it is not treated initially the infection spread from the tooth to surrounding tissues net result in an edentulous mouth. Penicillin and erythromycin are the standard drugs generally prescribed for prevent dental caries in humans, but nowadays they are rarely used clinically due to its adverse effects. Recently natural remedies from medicinal plants are good alternative for antibiotic and are free from hypersensitivity reaction, supra infections and teeth staining (Duraipandiyan, 2007; Shyla, 2011; Sevindik et al., 2017; Mohammed et al., 2018; Mohammed et al., 2019) . The study results suggest that chloroform extract showed significant antibacterial activity in all the assay methods moreover it showed the potency which is equivalent to standard antibiotics Penicillin and Amphotericin B.

Droserae Herba a formulation contains dried plant of *Drosera* (aerial part) in the form of extracts used for the treatment of various ailments. *D. rotundifolia* also a carnivorous plant it's ethanol and aqueous extracts inhibits neutrophil elastase in human and also showed antispasmodic effect on guinea pig ileum experiment. *D. peltata* extracts antimicrobial activity was studied on oral bacteria by agar diffusion in which chloroform extract showed highly significant antimicrobial effect (Duraipandiyan, 2007; Singh 2007; Shyla, 2011). Similarly, Indian *D. peltata* J.E.Sm. also exhibited significant antibacterial effects on clinically isolated human dental caries. The orders of antimicrobial effects were CEDP> EEDP> AEDP. Its antibacterial effect might be their bioactive compounds such as flavonoids and naphthoquinones such as plumbagin.

Conclusion

Bacterial resistance to antibiotics is an important issue which leads to development of newer and safer antimicrobials for prevention and treatment of oral infection. From the present study results it is evident that the plant *D. peltata* showed antimicrobial activity against dental caries such as *S. mutans* and *C. albicans.* It would be a therapeutically useful drug for the treatment of dental caries and other oral infections.

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Conflicts of Interest: No

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