

Anticariogenic Activity of *Gnidia glauca* (Fresen.) Gilg, *Pothos scandens* L. and *Elaeagnus kologa* Schlecht

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ABSTRACT

The present study was conducted to estimate total phenolic and flavonoid content and to determine anticariogenic efficacy of methanol extract of *Gnidia glauca* (Fresen.) Gilg (Thymelaeaceae), *Pothos scandens* L. (Araceae) and *Elaeagnus kologa* Schlecht (Elaeagnaceae) leaves. Total phenolic and flavonoid content of leaf extracts was determined by Folin-Ciocalteu method and Aluminium chloride colorimetric estimation method respectively. Anticariogenic activity of leaf extracts was assessed against 13 clinical isolates of *Streptococcus mutans* by Agar well diffusion technique. The contents of total phenolics and flavonoids were higher in *G. glauca* followed by *P. scandens* and *E. kologa*. The extract of *G. glauca* showed greater inhibition of cariogenic isolates than *P. scandens* and *E. kologa* as revealed by wider inhibition zones. The study revealed a correlation between the amount of phenolics and flavonoids present in the extracts and the anticariogenic activity of the extracts. The plants used in this study could be used against dental caries.

INTRODUCTION

Dental caries is a pathological, infectious, transmissible, localized and multifactorial disease that leads to the destruction of dental hard tissue. It requires three main factors viz., a susceptible host, cariogenic microflora and an adequate substrate. *Streptococcus mutans*, and to a lesser degree, *Streptococcus sobrinus* and species of *Lactobacillus* and *Actinomyces* are the major microorganisms associated with the incidence of dental caries. Development of resistance in cariogenic bacteria against antibiotics resulted in extensive screening of natural products, particularly from plants, for anticaries activity. Several studies have shown greater potential of plants against dental caries (Tellez et al., 2010; Tsai et al., 2008). *Gnidia glauca* (Fresen.) Gilg (Thymelaeaceae) is a large shrub. It is distributed in East Africa, peninsular India and Sri Lanka. Leaves alternate, 4-7x0.8-1.4 cm, linear-oblong, abruptly acute, sub-cordate; inflorescence is head subtended by involucre of bracts; flower bisexual, regular; calyx tubular, white hairy, circumscissile above ovary, 5 lobed,

imbricate, petals represented by 5 scales, inserted at throat of calyx tube sometimes O, ovary superior, 1-locular, ovule 1, pendulous, style lateral; fruit indehiscent, small, 1 seeded, enclosed by base of persistent calyx tube. Flowering and fruiting occur in January and February (Saldana and Nicolson, 1976). *Pothos scandens* L. (Araceae) is a climbing shrub with aerial roots growing on the trees and rocks like ivy. It is distributed in Western coast and Western Ghats up to 2500 feet.

Petioles broadly winged, wings truncate or rounded at the apex, narrowed to the semi-amplexicaul base, 1-2.5 inches long, 0.2-0.5 inches wide at the apex, in young shoots sometimes reduced to 0.2 inch long and 0.1 inch wide; blade of leaf usually lanceolate, sometimes linear-lanceolate, acuminate, rarely very broadly obovate and rounded, up to 4 inches long and 1.5 inches wide, often reduced to at mere point or altogether wanting, base rounded, veins close, forming a very acute angle with the mid-rib; peduncles axillary, solitary, 0.2-0.3 inch long, base embraced by a few minute, apiculate, suborbicular cataphylls; spathe suborbicular, apiculate, 0.1-0.2 inch long; spadix globose or abovoid, 0.25-0.3 inch long, deflexed; berries oblong, 0.5-0.7 inch long (Gamble, 1998a). *Elaeagnus kologa* Schlecht (Elaeagnaceae) is a large, sometimes thorny straggling or climbing shrub with orange red edible fruit.

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It is distributed in Western Ghats, Nilgiris and Pulney, margins of Shola woods and open scrub forests and Baba budan hills. Leaves medium sized, 2-3 inch long, 1.5-2 inch broad, thick, ovate or ovate-oblong or orbicular, obtuse or rarely acute at apex, usually rounded at base, 3-4 nerved; perianth tube broadly urceolate, 0.2-0.3 inch long; drupe oblong-ellipsoid, 0.5-0.7 inch long (Gamble, 1998b). Anticariogenic efficacy of these three plants has not been investigated so far. Hence, the present study was undertaken to determine total phenolic and flavonoid content and anticariogenic efficacy of methanol extract of leaves of *G. glauca*, *P. scandens* and *E. kologa*.

MATERIALS AND METHODS

Collection and identification of plant materials

The plants *G. glauca* (voucher number SRNMN/MB/SRDPVP-01), *P. scandens* (voucher number SRNMN/MB/SRDPVP-02) and *E. kologa* (voucher number SRNMN/MB/SRDPVP-03) were collected in and around Haniya, Hosanagara (taluk), Shivamogga, Karnataka and authenticated by Dr. Vinayaka K.S, Dept. of Botany, Indira Gandhi Government College, Sagara, Karnataka. Voucher specimens of the plants were deposited in the department for future reference.

Extraction

The leaves were separated, washed well, dried under shade and powdered mechanically using blender. A known quantity (100g) of powdered leaf materials were extracted with methanol in a Soxhlet extraction assembly. The extracts were filtered through 4-fold muslin cloth followed by Whatman No. 1 and concentrated in vacuum under reduced pressure and dried in the desiccator (Kekuda *et al.*, 2012).

Total Phenolic Content (TPC) of extracts

The TPC of extracts were determined by Folin-Ciocalteu (FC) reagent method employed by Kekuda *et al.* (2011) with minor modifications. Here, a dilute concentration of extracts (0.5 ml) was mixed with 0.5 ml of FC reagent (1:1) and 2 ml of sodium carbonate (7%). The reaction mixtures were allowed to stand for 30 minutes and the optical density was measured colorimetrically at 765nm. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000 µg/ml). The TPC of extracts was expressed as µg Gallic acid equivalents (GAE) from the graph.

Total Flavonoid Content (TFC) of extracts

The flavonoid content of extracts was estimated by Aluminium chloride colorimetric method (Zhishen *et al.*, 1999). A dilute concentration of extracts (0.5ml) was mixed with 0.5ml of methanol, 4ml of water, 0.3ml of NaNO₂ (5%) and incubated for 5 minutes at room temperature. After incubation, 0.3ml of AlCl₃ (10%) was added and incubated at room temperature for 6 minutes. 2ml of 1M NaOH and 2.4ml of distilled water were added and the absorbance was measured against blank (without

extract) at 510nm using UV-Vis spectrophotometer. A calibration curve was constructed using different concentrations of Catechin (standard, 0-120 µg/ml) and the flavonoid content of extracts was expressed as µg Catechin equivalents (CE) from the graph.

Anticariogenic activity of extracts

Thirteen clinical isolates of *Streptococcus mutans* (SM-1 to SM-13), recovered from infected teeth samples of dental caries patients were tested for their sensitivity to extracts. The isolates were maintained on sterile Brain heart infusion agar (HiMedia, Mumbai) slants in refrigerator. The isolates were inoculated into sterile Brain heart infusion broth (HiMedia, Mumbai) tubes and incubated overnight at 37°C and the broth cultures were used for susceptibility study by Agar well diffusion assay (Kekuda *et al.*, 2012). The isolates were aseptically swabbed on sterile Brain heart infusion agar plates using sterile cotton swabs. Using sterile cork borer, wells of 6mm diameter were punched in the inoculated plates and 100µl of extracts (50mg/ml of 10% DMSO), standard (Streptomycin, 1mg/ml) and DMSO (10%) were transferred into respectively labelled wells. The plates were incubated at 37°C aerobically for 24 hours and the zone of inhibition formed around the wells were measured. The experiment was repeated twice and the average value was recorded.

Statistical analysis

The data were presented as Mean ± Standard deviation (SD) of the number of experiments (3). Past software version 1.92 was used.

RESULTS AND DISCUSSION

TPC and TFC of leaf extracts

TPC and TFC in methanol extracts of leaves were estimated by Folin-Ciocalteu and Aluminium chloride colorimetric method respectively. The contents of both total phenolics and flavonoids were higher in *G. glauca* followed by *P. scandens* and *E. kologa* (Table 1).

Table. 1: TPC and TFC of leaf extracts.

Extract	TPC (µg GAE/mg, Mean±SD)	TFC (µg CE/mg, Mean±SD)
<i>G. glauca</i>	126.25±0.20	25.75±0.10
<i>P. scandens</i>	60.05±0.30	5.25±0.05
<i>E. kologa</i>	43.75±0.25	3.75±0.30

Anticariogenic activity leaf extracts

Inhibitory efficacy of leaf extracts against the clinical isolates of *S. mutans* was investigated by Agar well diffusion method. The effect of extracts was observed as the zone of no growth of cariogenic isolates around the well.

Among extracts, extract of *G. glauca* inhibited the cariogenic isolates to higher extent followed by *P. scandens* and *E. kologa*. Inhibition produced by standard antibiotic (Streptomycin) was higher than all three leaf extracts. The inhibition zone was found to be 1.6 to 2.6, 1.1 to 1.9 and 0.8 to 1.5 cm in case of *G.*

glauca, *P. scandens* and *E. kologa* respectively. There was no inhibition in case of DMSO which was used to prepare extracts (Table 2).

Table. 2: Anticariogenic activity of leaf extracts.

Isolates	Zone of inhibition in cm (Mean±SD)			
	<i>G. glauca</i>	<i>P. scandens</i>	<i>E. kologa</i>	Streptomycin
SM-1	2.0±0.21	1.5±0.10	1.3±0.20	2.9±0.10
SM-2	1.6±0.10	1.1±0.10	0.8±0.22	2.7±0.10
SM-3	2.4±0.12	1.8±0.12	1.5±0.30	3.1±0.20
SM-4	2.0±0.22	1.4±0.20	1.2±0.20	2.8±0.20
SM-5	2.1±0.14	1.7±0.15	1.3±0.30	3.1±0.30
SM-6	2.3±0.12	1.5±0.20	1.4±0.24	3.4±0.10
SM-7	2.0±0.10	1.4±0.11	1.5±0.22	2.6±0.20
SM-8	1.9±0.33	1.6±0.10	1.2±0.16	2.3±0.10
SM-9	2.4±0.24	1.9±0.32	1.1±0.10	3.0±0.10
SM-10	2.0±0.20	1.6±0.30	0.9±0.17	2.7±0.20
SM-11	1.8±0.20	1.8±0.20	1.2±0.16	2.6±0.10
SM-12	2.2±0.10	1.5±0.30	1.4±0.12	3.2±0.10
SM-13	2.6±0.21	1.7±0.33	1.1±0.12	3.5±0.30

Dental caries is a transmissible infectious disease of oral cavity in which microorganisms play a significant role and affects people of all age groups. The microflora in dental caries is highly complex and varies among individuals. The composition of the dominant groups may depend on diet, saliva, and the chronicity of the lesion. Mutans group streptococci, such as *S. mutans* and *S. sobrinus*, and lactobacilli are important bacteria which are implicated in the initiation and progression of disease. Mutans Streptococci are generally considered to be the principal etiological agent of dental caries. These microorganisms are acidogenic (acid producers) and results in the demineralization of enamel and dentin by fermenting dietary carbohydrates. They are also aciduric (acid tolerant), which gives competitive survival advantage (Napimoga *et al.*, 2005; Hahn and Liewehr, 2007; Almeida *et al.*, 2012). Antibiotics are significant in the control of infection in modern dentistry. However, development of resistance in cariogenic bacteria to various antibiotics makes it necessary to select agents useful for successful therapy from other sources such as plants. It was observed that herbal products used against oral infection have marked inhibitory effect on dental pathogens and various plants and extracts are used traditionally for dental care. Plants such as babul, neem, clove and many others are used in India for brushing teeth and traditional dental care in India (Sweeney *et al.*, 2004; Pathak *et al.*, 2012).

Plants and plant extracts contain a variety of phytochemicals with biological properties that promote human health and help reduce the risk of chronic diseases. Drugs from plants have a long history in both traditional and modern societies as herbal remedies or crude drugs and as purified compounds. The active principles of plants are also used as starting materials for further modifications. The drug discovery from plants still provides important new drug leads and many of which have potential clinical uses (Conforti *et al.*, 2008). Plants have the ability to produce a variety of secondary metabolites, most of which are phenols or their oxygen-substituted derivatives (Cowan, 1999). Phenolic compounds are widely present in the plant kingdom (>8,000 known) and have different chemical structures

and activities. They are found in fruits, vegetables, nuts, seeds, tea, red wine, citrus fruits and others and are consumed regularly as part of diet. The main classes of phenolic compounds are phenolic acids, flavonoids, lignans, stilbenes, coumarins, and tannins. Phenolic compounds have a wide range of activity such as antitumor, antioxidant, antiviral, antibacterial, cardioprotective, and antimutagenic activities (Yanez *et al.*, 2004). Flavonoids are naturally occurring polyphenolic compounds present in fruits, vegetables, beverages and various dietary supplements. They are potential in preventing various diseases, such as cardiovascular disease, inflammatory disorders, viral infections, diabetes and neurological conditions (Androutsopoulos *et al.*, 2010).

The inhibitory efficacy of plants against microorganisms has been linked with the presence of metabolites such as phenolic compounds, alkaloids, terpenoids, saponins and others. Many studies have revealed the inhibitory activity of phenolic compounds and other phytochemicals. The phenolic fractions of aerial part of *Scrophularia frutescens* and *S. sambucifolia* have shown potent antibacterial activity (Fernandez *et al.*, 1996).

In a study by Silva *et al.* (2010), the phenolic compounds isolated from aqueous fraction of the latex of *Himatanthus sucuuba* showed antimicrobial activity. Tenore *et al.* (2011) have shown antioxidant and antimicrobial properties of polyphenolic fractions from selected Moroccan red wines. Ali *et al.* (2011) showed antioxidant and antimicrobial activities of phenolic compounds extracted from five species of *Hypericum* showed. In a study, Siddiqi *et al.* (2011) observed significant antimicrobial activity of polyphenolic fractions derived from *Grewia asiatica*, *Eugenia jambolana* and *Carissa carandas*. Yano *et al.* (2012) have shown the efficacy of phenolic fraction from the pomace of *Vitis coignetiae* on biofilm formation by *S. mutans*. Cueva *et al.* (2012) showed antibacterial effect of seven wine phenolic compounds and six oenological phenolic extracts on the growth of pathogenic bacteria associated with respiratory diseases. Konate *et al.* (2012) observed *in vitro* antibacterial activity of polyphenol-rich fractions from *Sida alba*. The active flavonoid compound, quercetin-3-O-alpha-l-arabinopyranoside (guaijaverin) isolated from *Psidium guajava* demonstrated high potential antiplaque agent by inhibiting the growth of the *S. Mutans* (Prabhu *et al.*, 2006). The saponin fraction of *Madhuca longifolia* and *Bauhinia purpurea* were shown to possess marked inhibition of oral pathogens namely *S. mutans*, *Lactobacillus acidophilus* (Jyothi and Seshagiri, 2012). In our study, the inhibitory effect of extracts was found to correlate with the TPC and TFC. The extracts of all the three plants inhibited the growth of *S. mutans* isolates.

CONCLUSION

In our study, the anticariogenic activity was higher in *G. glauca* followed by *P. scandens* and *E. kologa*. The extent of inhibition of cariogenic isolates could be correlated with the phenolic and flavonoid contents present in the extracts as the content of total phenolics and flavonoids were higher in *G. glauca*

followed by *P. scandens* and *E. kologa*. The plants used in this study can be used for treatment of dental caries. Further studies on isolation of active compounds and their efficacy testing are under progress.

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