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Contents of total phenolics and flavonoids, radical scavenging and anticaries activity of leaf and seed extract of *Anisomeles indica* Linn

Syed Junaid [1],

Dileep N [1],

Rakesh K.N [1],

Prashith Kekuda T.R [1],

Raghavendra H.L [2*]

[1]Department of Microbiology, SRNMN College of Applied Sciences, NES Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, India
[2]College of Medical and Health Sciences, Wollega University, Post Box No: 395, Nekemte, Ethiopia

Corresponding Authors:
Raghavendra H L
Email: raghu_hl@rediffmail.com

Abstract:

The present study was carried out to determine the radical scavenging and anticaries activity of methanol extract of leaf and seed of *Anisomeles indica* Linn. (Lamiaceae). Total phenolic and flavonoid content of leaf and seed extract were determined by Folin-Ciocalteau Reagent method and Aluminium chloride colorimetric estimation method respectively. Radical scavenging activity of different concentrations of leaf and seed extracts was evaluated by DPPH free radical scavenging assay. Anticaries activity of leaf and seed extracts was performed against six clinical isolates of *Streptococcus mutans* by Agar well diffusion assay. The content of total phenolics and flavonoids were higher in leaf extract when compared to seed extract. Both the extracts scavenged DPPH radical in a dose dependent manner. Leaf extract was more efficient in scavenging radicals (IC50 2.70µg/ml) than seed extract (IC50 8.18µg/ml). Similarly, leaf extract inhibited *S. mutans* isolates to higher extent than seed extract. A marked radical scavenging and anticaries activity of leaf and seed extract of *A. indica* was observed in this study. Leaf extract was more efficient in scavenging DPPH radicals and inhibiting the *S. mutans* isolates. The high phenolic and flavonoid content of leaf extract might be attributed to higher radical scavenging and antibacterial activity.

Keywords: *Anisomeles indica*, Dental caries, *Streptococcus mutans*, DPPH, Phenolics, Flavonoids.

Introduction

Anisomeles indica Linn. belonging to the family Lamiaceae is a medicinal plant being used ethnomedicinally in various parts of the world [1,2,3]. Crude extracts, essential oils and purified compounds from various parts of the plant such as roots, leaves and flowers have shown to possess marked biological activities such as antimicrobial [4-8], attenuation of inflammation [7], analgesic [10], anti-inflammatory [11], antiviral [12], antiplatelet aggregation activity [13] and others. In the present study, we have estimated the content of total phenolics and flavonoids and evaluated radical scavenging and anticaries activity of methanol extract of leaves and seeds of *A. indica*.

Materials and Methods

Collection and identification of plant material

The plant *A. indica* was collected from the outskirts of Shivamogga city and identified by Dr. Vinayaka K.S, Lecturer, Dept. of Botany, Indira Gandhi Government College, Sagara-577401, Karnataka, India. The leaves and seeds were dried and powdered.

Extraction

The powdered leaves and seeds were subjected to soxhlet extraction and extracted with methanol (HiMedia, Mumbai). The extracts were then filtered through Whatman No. 1 filter paper and concentrated in vacuum under reduced pressure and dried in the desiccator [14].

Total Phenolic Content (TPC) of leaf and seed extract

The TPC of leaf and seed extract was determined by Folin-Ciocalteau reagent (FCR) method. A dilute concentration of extract (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) and the TPC of extracts was expressed as µg Gallic acid equivalents (GAE) from the graph [14].

Total Flavonoid Content (TFC) of leaf and seed extract

The flavonoid content of leaf and seed extract was estimated by Aluminium chloride colorimetric method. A dilute concentration of extract (0.5ml) was mixed with 0.5ml of methanol, 4ml of water, 0.3ml of $NaNO_2$ (5%) and incubated for 5 minutes at room temperature. After incubation, 0.3ml of $AlCl_3$ (10%) was added and again 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, 0-120µg/ml) and the TFC of extracts was expressed as µg Catechin equivalents (CE) from the graph [14].

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of leaf and seed extract

The radical scavenging ability of leaf and seed extract was tested on the basis of the radical scavenging effect on the DPPH free radical. Briefly, 2 ml of different concentrations of leaf and seed extract was mixed with 2 ml of DPPH solution (0.004% in methanol) in separate tubes. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517 nm using UV-Vis spectrophotometer. The absorbance of the DPPH control was also noted. Ascorbic acid was used as reference standard. The scavenging activity of the extracts was calculated using the formula:

Scavenging activity (%) = [(Ao - Ae) / Ao] x 100, where Ao is absorbance of DPPH control and Ae is absorbance of DPPH and extract/standard combination [14]. The IC50 value for each of the extracts was calculated. IC50 denotes the concentration of extract required to scavenge 50% of free radicals.

Anticaries activity of leaf and seed extract

Inhibitory effect of leaf and seed extract was evaluated against a panel of six isolates of *Streptococcus mutans* recovered from dental caries patients by Agar well diffusion assay [15]. The bacterial isolates were maintained on sterile Brain heart infusion agar (HiMedia, Mumbai) slants were inoculated into sterile Brain heart infusion broth (HiMedia, Mumbai) tubes and incubated overnight at 37°C. The broth cultures were aseptically swabbed on sterile Brain heart infusion agar plates using sterile cotton swabs. Using a sterile cork borer, wells of 6 mm diameter were punched in the inoculated plates and 100µl of leaf and seed extract (25mg/ml of 10% dimethyl sulfoxide [DMSO]), standard (Streptomycin, 1mg/ml) and DMSO (10%) were transferred into respectively labeled wells. The plates were incubated at 37°C for 24 hours and the zones of inhibition formed around the wells were measured.

RESULTS AND DISCUSSION

Polyphenolic compounds from various parts of the plants are the most effective antioxidative constituents. It is important to estimate phenolic contents of extracts of plants in order to assess their contribution to antioxidant activity [16]. In the present study, total phenolic content of leaf and seed extract was estimated by FCR method. Leaf extract was found to contain more phenolic content than seed extract (Table 1). The method is one of the oldest and widely used colorimetric techniques for the estimation of total phenolics of a variety of samples including plant extracts. Phenolic compounds reacts with FCR under basic conditions (adjusted by sodium carbonate solution to pH 10) to form blue complex having maximum absorption near 750nm. Though the chemical nature of FCR is undefined, the total phenols estimation by FCR is convenient, simple, and reproducible. As a result, a large data has been accumulated, and it has become a routine assay in studying the phenolic antioxidants [14,17-24].

Flavonoids are polyphenolic compounds accounting for majority of secondary metabolites of plants. These compounds have shown to possess remarkable health promoting effects mainly antioxidant activity [25]. In this study, we estimated flavonoid content in leaf and seed extract of *A. indica* by aluminium chloride colorimetric estimation method. Flavonoids content was found to be higher in leaf extract when compared to seed extract (Table 1). Aluminium chloride colorimetric estimation is a widely used method to estimate flavonoid content of plant extracts [26-28]. Total flavonoid contents can be estimated by reaction with sodium nitrite, followed by the development of colored flavonoid-aluminium complex formation using aluminium chloride in alkaline condition which can be monitored spectrophotometrically at wavelength of 510 nm [28].

Table 1: Total phenolic and flavonoid content of leaf and seed extract

Extract	TPC (µg GAE/mg)	TFC (µg CE/mg)
Leaf	106.68	30.80
Seed	76.65	22.50

DPPH free radical scavenging activity of leaf and seed extract

Antioxidants are substances that inhibits or delay oxidative damage when present in small quantities compared to an oxidizable substrate. Antioxidants effectively quench free radicals or inhibit damage caused by them and thus help in disease prevention. Endogenous antioxidants (ascorbic acid, vitamin E and others) act as primary defense system protecting against oxidative damage. However, in pathophysiological conditions, there is an extra need for antioxidants from exogenous sources such as food and medicinal plants [29]. In the present study, we evaluated antioxidant activity of leaf and seed extract of *A. indica* by DPPH free radical scavenging assay. DPPH is a stable organic nitrogen centred free radical having maximum absorption at 517nm in methanolic solution. On accepting an electron or hydrogen atom, this radical becomes a stable diamagnetic molecule [30]. The assay of DPPH free radical scavenging is one of the most widely used *in vitro* assays for determining the radical scavenging effect of various kinds of samples including plant extracts. In the presence of an extract/compound with hydrogen atom donating capacity, the free radical nature of DPPH is lost and its color (purple) changes to yellow (diphenylpicrylhydrazine) [14,16,17,20,22-24,30-33]. In this study, a decrease in DPPH

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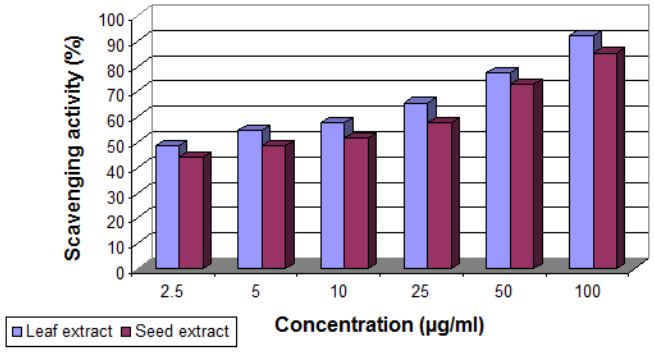
absorption in the presence of varying concentrations of leaf and seed extracts was monitored at 517nm and the result is shown in Figure 1. Both the extracts showed dose dependent scavenging of DPPH free radicals. Among extracts, scavenging potential was greater in leaf extract (IC50 2.70 µg/ml) than seed extract (IC50 8.18 µg/ml). The scavenging effect of ascorbic acid (IC50 2.27µg/ml) was higher than that of leaf and seed extracts. A positive correlation was observed between the total phenolic and flavonoid content of extracts and the radical scavenging activity. Leaf extract contained high total phenolics and flavonoids and displayed marked scavenging potential. The result obtained is in justification with earlier studies where extracts/plants containing high phenolics displayed high scavenging potential [14,20,23,33-36]. Although the scavenging abilities of extracts were lesser than that of ascorbic acid, it was evident that the extracts possess hydrogen donating ability and therefore the extracts could serve as free radical scavengers, acting possibly as primary antioxidants [19].

Figure 1: DPPH free radical scavenging activity of leaf and seed extract



Anticaries activity of leaf and seed extract

Oral diseases *viz.*, dental caries, periodontal diseases and teeth loss influence greatly the human health. Dental caries is one of the most prevalent infectious diseases affecting people of all age groups throughout the world and is caused by the interaction among oral microflora, diet, dentition and oral environment. This chronic disease affects 60-90% of young population. Microorganisms such as mutans streptococci, lactobacilli and *Actinomyces* have been implicated in causing dental caries. Among these, *Streptococcus mutans* is considered to be the main aetiological agent. The cariogenic ability of this pathogen is attributed to its ability to metabolize dietary carbohydrates, to adhere to tooth surfaces and to form biofilm on tooth surfaces [37-40]. The cost of synthetic antimicrobials, their possible side effects and the development of resistance in caries causing bacteria stimulated researchers to screen a variety of plants for developing novel antimicrobials with anticaries activity [15,41-43]. In the present study, we have determined anticaries activity of methanolic extract of leaf and seed extract of *A. indica* against six clinical isolates of *S. mutans* recovered from dental caries subjects and the result is shown in Table 2. Both the extracts were effective in inhibiting the clinical isolates of *S. mutans*. Leaf extract was more effective against the bacterial isolates (zones of inhibition 2.3 to 3.0cm) than seed extract (zones of inhibition 1.6 to 2.3cm). However, the inhibitory effect of extracts was lesser when compared to standard antibiotic. DMSO did not cause inhibition of cariogenic isolates.

Table 2: Anticariogenic activity of leaf and seed extract

Test bacteria	Zone of inhibition in cm		
	Leaf extract	Seed extract	Streptomycin
SM-1	2.4	2.1	3.4
SM-2	2.9	2.3	3.8
SM-3	2.6	2.0	3.1
SM-4	2.9	1.8	2.9
SM-5	2.3	1.6	3.1
SM-6	3.0	2.3	3.9

Conclusion

In the present study, a marked radical scavenging and anticaries activity of leaf and seed extract of *A. indica* was observed. Leaf extract was more effective in scavenging free radicals and inhibiting clinical isolates of *S. mutans* when compared to seed extract and the potential of leaf extract might be attributed to the high phenolic and flavonoid content.

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