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Comparative phytochemical composition and antimicrobial potential of leaf and twig extracts of *Ginkgo biloba* L. from India

Geeta Tewari[1*], Brijmohan[2], Lalit Tewari[2], Kamal Kishor[2], Tapan K. Nailwal[3] and Manoj Singh[3]

[1]Department of Chemistry, [2]Department of Botany, D. S. B. Campus, Kumaon University, Nainital, Uttarakhand, INDIA
[3]Department of Biotechnology, Bhimtal Campus, Kumaon University, Bhimtal, Uttarakhand, INDIA

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ABSTRACT

Antimicrobial potential of leaf and twig extracts of Himalayan gymnosperm *Ginkgo biloba* L. has been determined by agar well diffusion and agar dilution methods respectively. Strains of five Gram positive bacteria were used as test organisms. Plant extract were prepared in different organic solvents (methanol, hexane and DCM). All plant extracts exhibited antimicrobial activity against all these tested bacterial strains. Methanolic extract of leaf and twig, and hexane leaf extract of *G. biloba* showed maximum activity against E. coli with Minimum Inhibitory Concentration (MIC) 2.01, 2.53 and 2.97 mg/mL, respectively.

1) INTRODUCTION

The steadily increasing drug resistant microorganisms such as bacteria and fungi are now a therapeutic challenge. Immuno-deficient persons have higher risk of such infections [1, 2]. Some microbial infections required prolonged therapy, resulting various side effects, become a serious concern particularly in developing countries due to unavailability of medicines, their high cost, and increasing antibiotic resistance in pathogenic microbes. Therefore, the search for new naturally occurring antimicrobial substances from medicinal plants is gaining importance. Plant originated compounds are still proving to be an invaluable source of medicines for humans. The use of plants by humans in both traditional and modern medicinal systems, therefore, largely exploits these plant-based principles [3]. Thus use of medicinal plants, on the basis of their ethno-medicinal uses, and their efficacy as new drugs becomes new research area now days [4].

The living fossil *Ginkgo biloba* L. (Ginkgoaceae) is the only living member of this family [5]. *G. biloba*, being a native of China, was also distributed in Indian Himalayan region at an altitude of 1800 – 2000m. *G. biloba* has been known for its valuable groups of medicinally important compounds, such as terpenoids, flavonoids, glycosides, polyphenols, organic acids and amino acids [6, 7].. Flavonoids, glycosides and terpenoids are considered to be the most bioactive class of constituents in *G. biloba* [8]. This plant has been traditionally used for the treatment of cardiovascular and neurological diseases, while its leaf extract is used in cosmetics and skin care products. There are only few reports on its essential oil composition [9, 10, 11, 12], extract composition [13, 14] and antimicrobial activities [15, 16]. Therefore, the present work is carried out to investigate the comparative phytochemical composition and antibacterial potential of the leaf and twig extracts of *G. biloba* from Uttarakhand, India.

2) MATERIALS AND METHODS

***2.1 Plant material*:** Fresh plant samples of *Ginkgo biloba* were collected from Nainital (Uttarakhand, India) in May 2014. The plant was identified by Prof. Y.P.S. Pangtey, Botany Department, Kumaun University, Nainital, and deposited in Botanical Survey of India, Dehradun for future reference (Acc. No. 10359).

2.2 Phytochemical investigation: The fresh plant samples were washed thoroughly with water and dried under shade and crushed into coarse powder for extraction. Extracts were prepared by mixing 50 g of powdered plant material (leaves and twig, respectively) to 200 mL of three organic solvents with different polarity {methanol, hexane and dichloromethane (DCM)} separately, in triplicate. Each extract was shaken and after six hours, filtered through Whatman filter paper 42. The extract yield was calculated (%, w/w). Phytochemical screening for steroids, glycosides, carbohydrates, flavonoids, terpenoids, tannins and saponins were carried out as by standard methods [17].

2.3 Bacterial strains: Five Gram positive bacterial strains: *Escherichia coli* (MTCC443), *Salmonella typhimurium* (MTCC3224), *Pseudomonas aeruginosa* (MTCC424), *Pseudomonas fluorescens* ((MTCC103)) and *Proteus vulgaris* (MTCC1771) used were obtained from Institute of Microbial

* Corresponding Author: Dr. Geeta Tewari
Email address: geeta_k@rediffmail.com

Technology, Chandigarh.

***2.4 Chemicals*:** All chemicals used in phytochemical investigation were purchased from Merck, India and chemicals used in antibacterial activity were purchased from HiMedia Laboratories, India.

***2.5 Agar well diffusion method*:** To test the antibiotic sensitivity against the bacterial strains, a preliminary evaluation of the antibacterial activity of the leaf and twig extracts were determined by the agar well diffusion method using the well technique proposed by the Clinical and laboratory Standards Institute [18]. Briefly, all the samples were dissolved in their respective solvents in order to obtain desired concentration (15mg/mL). Inoculums of the microbial strains ($1X10^6$ CFU/mL) were then plated using sterile swabs into petri dishes (90 mm) with 20 mL of Mueller–Hinton agar, where 3 mm wells were cut and filled with 20 µL of extract of 1 mg/mL. Ampicillin was used as standard in antibacterial assay. The solvents were used as positive control and showed no activity. The petri dishes were pre-incubated for 3 h at room temperature, allowing the complete diffusion of the sample [19] and then incubated at 37 ± 1^0C for 24h. The complete diffusion of samples in the Mueller–Hinton agar was examined visually and confirmed. The antimicrobial activities of the samples were evaluated by measuring the inhibition zones. The absence of zone inhibition was interpreted as the absence of activity [20]. All experiments were carried out in triplicates under strict aseptic conditions.

***2.6 Minimum inhibitory concentrations (MIC*):** Minimum Inhibitory Concentration was determined by agar dilution method [21]. Different concentrations of methanol, hexane and DCM extracts of leaf and twig of *Ginkgo biloba* from 0.125 to 15 mg/mL (0.125, 0.250, 0.5, 1.0, 2.0, 4.0, 8.0 and 15 mg/mL) were prepared. The extracts were mixed thoroughly with 14 mL of autoclaved nutrient agar (NA) medium and poured into sterile petri-plates and were allowed to solidify. Growth control was prepared by inoculating 1mL of each culture suspension on 14 mL NA medium without any extract or solvent (drug-free medium). Solvent control was prepared by pouring 1mL of solvent to 14 mL of NA medium followed by seeding of cultures. The plates were allowed to diffuse for 1h and incubated at 37ºC for 24 h. Minimum inhibitory concentration (MIC), was determined as the lowest concentration of antimicrobial agent that inhibited the visible growth of a microorganism after overnight incubation.

***2.7 Statistical analysis*:** For all the observations, the mean values and standard deviations were calculated. The data were analyzed by one-way Analysis of Variance (ANOVA) and the means were compared by Duncan tests at a level of significance of $p < 0.05$ using SPSS 16.0 statistical software.

3) RESULTS AND DISCUSSION

The yield of the methanolic extract of was found to be highest (5.64%±0.09), while the hexane extract of the leaf showed lowest **(Table 1)**. The methanol, hexane and DCM extracts of leaf and twig of *G. biloba* were analyzed for the presence of tannins, flavonoids, saponins, carbohydrates, steroids, glycosides and terpenoids. The phytochemical composition of *Ginkgo biloba* L. showed positive and negative results for different extracts **(Table 2)**. Only saponins, were absent in twig extracts. Antibacterial activity of *Ginkgo biloba* methanol, hexane and DCM extracts against five Gram positive bacterial strains were evaluated in present study and their potent activity were quantitatively assessed by zone of inhibition (ZI) **(Table 3)** and MIC **(Table 4)**.

Table 1: Yield of *G. biloba* leaf and twig extracts

Extracts	Leaf (%)	Twig (%)
Methanol	5.64±0.09 [c]	4.50±0.08 [c]
Hexane	0.77±0.06 [b]	1.50±0.06 [b]
DCM	0.59±0.06 [a]	1.00±0.06 [a]

Table 2: Phytochemical composition of *Ginkgo biloba* L. extracts

Chemical Constituents	Methanol Extract		Hexane Extract		DCM Extract	
	Leaf	Twig	Leaf	Twig	Leaf	Twig
Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Saponins	+	_	+	_	+	_
Carbohydrates	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+

(+)Present/ (-) Absent

Methanol extract of *G. biloba* L. showed excellent antibacterial growth inhibition. Leaf extracts had more potent activity as compared to twig extracts. *E. coli* (16.00 ±0.06 mm) was the most sensitive bacteria followed by *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *P. flurescens* and *Proteus vulgaris*. The MIC values for leaf methanol extract was the lowest i.e. 2.01 mg/mL.

The hexane extracts of *G. biloba* also showed excellent activity against all tested bacteria. The highest inhibition was observed for *E. coli* (ZI = 16.40±0.11 mm; MIC = 2.97 mg/mL) followed by *Pseudomonas aeruginosa*, *P. vulgaris*, *P. fluorescens* and *Salmonella typhimurium*.

The DCM extract was more effective against *E. coli*, *P. aeruginosa* and *P. fluorescens*. The MIC values ranged from 3.51 to 8.19 mg/mL. According to Sati and Joshi [15], the methanol extract of *G. biloba* was the most active among all the examined solvent extracts. Another report on antimicrobial activity of *G. biloba* leaf extracts also suggested that methanol extract gave the best activity results [16]. The presence of saponins could be responsible for activity leaf extract of *G. biloba.*

4) CONCLUSION

The present study indicates that all the extracts have excellent activity against all tested bacterial strains especially *E. coli*. Leaf extract was more potent as compared to twig extract.

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Table 3: Antibacterial activity of *G. biloba* extracts evaluated by agar well diffusion method

Bacterial strains	Standard	Methanol extract ZI (mm)		Hexane extract ZI (mm)		DCM extract ZI (mm)	
	mpicillin ZI (mm)	Leaf	twig	Leaf	twig	Leaf	Twig
E. coli	7.03±0.09[c]	16.00±0.06[e]	16.40±0.09[e]	16.40±0.11[e]	13.18±0.10[d]	15.47±0.22[e]	10.77±0.09[e]
Salmonella typhimurium	9.53±0.29[d]	14.81±0.08[d]	13.62±0.09[d]	10.03±0.08[a]	8.79±0.06[c]	9.01±0.06[b]	8.80±0.06[b]
Pseudomonas aeruginosa	6.72±0.14[b]	12.57±0.15[c]	11.17±0.09[c]	13.60±0.12[d]	13.21±0.05[e]	11.41±0.07[d]	9.21±0.07[c]
Pseudomonas fluorescens	5.03±0.26[a]	11.01±0.07[b]	7.51±0.05[a]	10.51±0.04[b]	8.01±0.05[a]	8.81±0.09[a]	7.21±0.07[a]
Proteus vulgaris	9.77±0.12[e]	9.63±0.10[a]	8.80±0.10[b]	13.45±0.09[c]	8.59±0.07[b]	9.44±0.07[c]	9.43±0.07[d]

Table 4. Minimum Inhibitory Concentration (MIC) of *Ginkgo biloba* extracts

Bacterial strains	Leaf extracts MIC (mg/mL)			Twig extracts MIC (mg/mL)		
	Methanol	Hexane	DCM	Methanol	Hexane	DCM
E. coli	2.01	2.97	3.52	2.53	3.51	4.06
Salmonella typhimurium	2.50	3.10	4.04	3.11	3.78	4.16
Pseudomonas aeruginosa	3.48	4.01	4.60	4.98	5.40	6.00
Pseudomonas fluorescens	4.50	4.77	4.97	6.50	7.02	7.20
Proteus vulgaris	5.02	5.18	5.68	7.01	7.59	8.19

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