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Research Article

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Investigation the Antimicrobial Activity of Methanol Extracts of the Leaves of *Ficus Nitida*

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Abstract

The aim of this study was to prepare crude extracts from the leaves of Ficus nitida and determine their content as well as their antimicrobial activity. Powdered leaf samples were extracted with methanol by using maceration method. The resulting crude extract was suspended in water and successively extracted with petroleum ether, chloroform, ethyl acetate and methanol solvents respectively. Total flavonoids of the above extracts were determined by the aluminum chloride method and antimicrobial activity of the same extracts was assessed by a slightly modified disc diffusion method against different gram positive and gram negative bacterial strains. The highest flavonoids content was found in chloroform crude extract followed by that of petroleum ether, ethyl acetate and methanol extracts respectively. Tested against four bacterial strains, the extracts showed activity in the range of 0-12 mm, using amoxicillin as standard. The present findings could establish this plant as a potential source of antibiotics.

Keywords: *Ficus Nitida*, Maceration Extractor, Amoxicillin Antimicrobial Activity

Introduction

Ficus nitida L. (moraceae) is a multipurpose tree found in various parts of the world. *F. nitida* is native to a large area including india, southern china, south east asia, malaysia, philippines, northern australia and the islands of the south pacific [1]. *F. nitida* is cultivated in many parts of the world including american samoa (tutuila), french polynesia (cult.), marshall islands (kwajalein (cult.), majuro (cult.), tonga as well as florida in the united states [2]. It grows as a large evergreen shrub, up to 6 m tall, with nearly 8 m wide spreading crown and drooping shoots with young slender twigs. The plant is well known due to its medicinal potential. Its latex and some fruit extracts are used by indigenous communities to treat skin disorders, inflammation, piles, vomiting, leprosy, malaria, nose-diseases and cancer besides the use as a general tonic. The plant is also used as antimicrobial, antinociceptive, antipyretic, hypotensive and anti-dysentery remedy. The leaves and twigs are used as insect repellant [3-6]. The leaves, bark and fruits of *F. nitida* contain various bioactive constituents like cinnamic acid, lactose, catechin, morin, rutin, naringenin, quercetin, caffeic acid and stigmasterol [5]. The chemistry and the biological properties of this plant will be available in the literature [7-10]. In the present study we conducted to evaluate some chemical and biological characteristics of *F. nitida* as an indigenous plant [11-21]. Therefore, the aim of this work was to prepare crude extracts from the leaves of *F. nitida* and determine their antimicrobial activity.

Materials and Methods

Preparation of Samples

The samples were washed with clean water and dried at room temperature under shade for two weeks and then ground into powder. The powdered samples were kept at low temperature in an amber color bottle for further processing.

Extraction Procedure

400 gm of leaves powder were mixed with 1 liter of methanol in a beaker and the mixture was held for 2 days after which it was filtered using Buckner funnel under pressure. Then the filtrate was evaporated by a rotary evaporator to obtain methanol crude extract. 40 grams methanol extract suspended in 250 ml distilled water were transferred into a separation funnel where the mixture was shaken manually with 50 ml of petroleum ether solvent for 30 min after which it separated into two layers; this process was repeated with 20 ml of petroleum ether. The petroleum ether phase in each treatment was separated and the amounts from both procedures were combined and evaporated to get the petroleum ether crude extract (8.4 gm). A similar procedure was applied to obtain

extracts from chloroform (4.82 gm), ethyl acetate (2.62 gm) and methanol (1.82 gm), respectively [12]. The remaining crude extracts part was evaporated to give the marc crude extract (7.15 gm).

Procedure for Total Flavonoids

The crude extracts of *F. nitida* were used for the determination of total flavonoids content. 0.25 ml of each sample (4 mg crude extract in 4 ml of methanol) was placed in a separate test tube. Then 150 µl water and 100 µl sodium nitrate solution were added to each tube followed by incubation at room temperature for 4 min in a dark place. 150 µl of aluminum chloride were then added to each tube with incubation in a dark place for 4 hours and finally 400 µl sodium hydroxide and 300 µl of water were added to each test tube. The absorbance was measured by UV-visible spectroscopy at fixed wavelengths of 510 nm.

Antimicrobial Activity

Various gram positive and gram negative bacterial strains were used for detecting antimicrobial activity by disc diffusion method [13]. Serial concentrations for each extract, 2 and 1 mg/ml were prepared using dimethyl sulphoxide (DMSO) and these were used to impregnate sterile filter paper discs (4 mm in diameter). For amoxicillin standard 4 mg were dissolved in 4 ml DMSO. Bacterial strains *E. coli, P.* spp., *S. aurus* and *H. influenza,* were used to determine the zone of inhibition for each dilutions of different extracts. All plates with their sets of discs were incubated micro aerobically at 40 °C for 24 hours and then the diameter of the zone of inhibition was measured for each disc against the test bacteria [13].

Results and Discussion

The different crude extracts from the powdered leaves of *F. nitida* prepared from the initial methanol extract were used for the determination of total flavonoids content and antimicrobial activity of each extract of *F. nitida* by aluminum chloride and slightly modified disc diffusion method against different selected gram positive and gram negative bacterial strains at different concentrations.

Total Flavonoids Content

Flavonoids are the most important group of natural phenols compounds. All of these compounds possess a broad spectrum of chemical and biological activities including antimicrobial activities. The total flavonoids content of the selected crude extracts of *F. nitida* was determined by aluminum chloride method with modification [12]. Quercetin was used as standard. Table 1 shows that the total flavonoids content of Petroleum ether, chloroform, ethyl acetate and methanol crude extract from leaves of *F. nitida*.

Table 1: Total Flavonoids Content of Different Leaves Crude Extracts of F. nitida

Crude extracts	Leaves mg QE/100 g dry plant material
Petroleum ether	530.72
chloroform	840.70
ethyl acetate	123.54
methanol	23.53

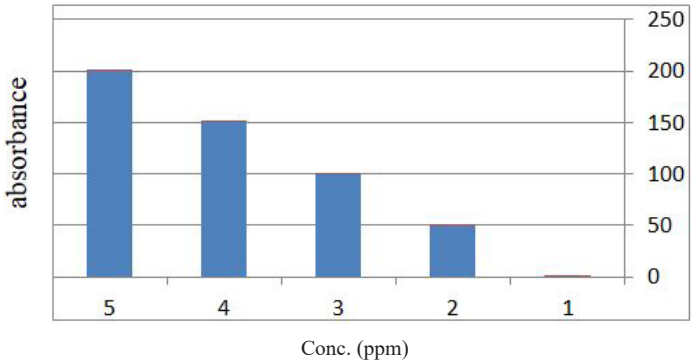

Figure 1: Quercetin Standard Curve

The results show that chloroform crude extract yielded the highest total flavonoids content (840.70 mg QE/100 g dry plant material) whereas the lowest amount obtained was in methanol crude extract (23.53 mg QE/100 g dry plant material). The yield of total flavonoids content was found to be in the order chloroform > Petroleum ether > ethyl acetate > methanol compared with previous studies.

Antimicrobial Activity

The antimicrobial activity of plant extract depends on dose and the type of bacterial strains used. In addition, the activity also depends on the chemical components present in the plant crude extracts. Generally, most of the crude extracts of plants contain steroids, saponins, tannins and flavonoids compounds [17, 18]. These compounds are also present in all crude extracts isolated from *F. nitida,* all of which may contribute to the antimicrobial activity of extracts in this study. The maximum antimicrobial activity indicates the maximum amount or concentration of active components present in the crude extract [16-20]. The in vitro antimicrobial activity of our extracts was estimated using standard conventional methods against *S. aureus, E. coli, H. influenza* and P. spp. [21, 22]. The presence or absence of inhibition zones of methanol extract and its subfractions was qualitatively assessed against the employed four selected bacterial strains. The methanol crude extract and its fractions were used against gram positive and gram negative bacterial strains at the concentration of 2 mg/ml and 1 mg/ml with respective zones of inhibition of 0-11 mm (Table 2). The different crude extracts of *F. nitida* exhibited strong antibacterial activity against one gram positive *S. aureus* and three gram negative E. coli, Haemophilus infleunza and Proteus. spp. bacteria at the above mentioned concentrations. Amoxicillin was used as a standard (Table 2).

Table 2: Antimicrobial Activity of Succesive Crude Extracts of the Leaves of *F. nitida*

Leaves crude extracts	Conc µg/ml	E. coli nm	P. spp. nm	S. aureus nm	H. infleunza nm
Pet. ether	2	9 ± 0.11	7 ± 0.10	9 ± 0.19	8 ± 0.10
	1	7 ± 0.17	6 ± 0.18	nd	7 ± 0.11
	Control	9 ± 0.16	7 ± 0.55	9 ± 0.43	8 ± 0.09
Chloroform	2	10 ± 0.27	7 ± 0.51	6 ± 0.10	6 ± 0.11
	1	9 ± 0.11	nd	nd	nd
	Control	9 ± 0.11	7 ± 0.10	8 ± 0.18	7 ± 0.19
Ethyl acetate	2	7 ± 0.19	7 ± 0.11	8 ± 0.11	nd
	1	6 ± 0.10	nd	7 ± 0.10	6 ± 0.44
	Control	9 ± 0.21	7 ± 0.12	10 ± 0.23	6 ± 0.10
Methanol	2	7 ± 0.08	7 ± 0.32	8 ± 0.17	nd
	1	nd	6 ± 0.17	7 ± 0.32	nd
	Control	9 ± 0.17	7 ± 0.11	10 ± 0.27	10 ± 0.11
nd = not detected					

The crude extracts of *F. nitida* at two different concentrations 2 and 1 mg/ml showed the zone of inhibition of 0-9 mm against *E. coli.* The highest activity was obtained from chloroform extract followed by ethyl acetate, petroleum ether and methanol. However, methanol crude extract at the concentration of 1 mg/ml did not showed any activity against *E. coli.* Similarly, all the crude extracts at two concentrations showed activity against *P. spp*. with zone of inhibition of 0-10 mm with the exception of chloroform and ethyl acetate extracts at concentrations of 1 mg/ml. Ethyl acetate and methanol crude extracts showed activity within the range of 0-12 mm at all concentrations employed against *S. aureus*. However, chloroform crude extract at the concentrations of 1 mg/ml did not show activity against *S. aureus* (Table 2). The petroleum ether crude extract showed the highest activity against *H. influenza*. The other crude extracts also showed activity against *H. influenza* but not including all the employed concentrations. The experimental results showed that in most cases the antimicrobial activity increased with increasing the polarity of the solvents. This probably depends on the active ingredient in the crude extract [23-26].

Conclusion

The antimicrobial activity and total flavonoids content of leaves crude extracts were estimated by the standard disc diffusion method against *S. aureus, E. coli, H. infleunza* and *P. spp.*, and aluminum chloride method. The antimicrobial activity and total flavonoids content results showed that all the crude extracts from the leaves of *F. nitida* gave strong antimicrobial activity and high total flavonoids content.

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