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Isolation and Identification of *Ficus Palmata* Leaves and Their Antimicrobial Activities

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Abstract

The aim of this work is to investigate functional compounds analysis and their antimicrobial activity of *Ficus palmata* leave. In this work some functional compounds were isolated by column chromatographic techniques and identification of their structure by spectroscopic (NMR, IR, Mass, UV, etc.) methods. Catechin, Genistein, β-Sitosterol and Stigmasterol compounds were isolated. These compounds firstly were isolated from *F. palmata* leave. The antimicrobial activity of these compounds has been investigated with some different bacterial stains. In this study, it has been shown that zone of inhibition power activities of these compounds showed potent antimicrobial activity as compared to standard drug.

Keywords: *Ficus palmate;* Isolation; Moraceae; Antimicrobial activity.

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1. Introduction

Ficus palmata (Moraceae) locally known as Bedu is a popular fruit among local people and abundantly distributed in the Garhwal Himalaya region [1]. The fruits of Bedu are consumed fresh or dried and its importance is not limited to its nutritive value but also their high contents of potentially health-promoting components. It is mostly consumed as fresh fruit in local markets but may also be dried, jam, jelly, squash, dry vegetable, pickled, and processed into marmalade, and fruits of Bedu are well known as traditional medicines in Uttarakhand and have been used for many years for the treatment of stomach ulcers, digestive system complaints [2], bronchitis, eczemas, haemorrhoids, and as a diuretic agent, among others [3]. *Ficus palmata* is employed frequently in the traditional medicine as gastrointestinal, hypoglycemic, insulinase, anti-tumour, anti-ulcer [4], anti-diabetic, lipid lowering, Anti-calcinogenic activity [5], antifungal activities and anti-inflammatory [6]. Although there are many studies on Bedu, the functional compound and

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antimicrobial activity of leave of *Ficus palmata* (Fig. 1) have not been well documented yet. The aim of this study was to evaluate isolation and structural identification of functional compounds in leave of Bedu and antimicrobial activity tests such as, the antimicrobial activity using the disc diffusion method. Results were compared with commercial and standard antimicrobial drugs such as Erythromycin commonly used by the food and pharmaceutical industry.

Fig. 1. Digital images of *Ficus palmata* leaves.

2. Experimental

2.1. *Chemicals & experimental instruments*

All the chemicals and reagents of analytical grade such as ethyl alcohol (Merck, Bangalore, India) and methanol (Himedia, Chemicals, Mumbai, India) were procured from the respective companies and were used in the study. Melting points were determined on a Kofler hot-stage microscope melting point apparatus and were uncorrected, infrared and ultraviolet spectra were obtained on Perkin-Elmer lambda model 1330 and 20 spectrometers respectively. ^{1}H- and ^{13}C-nuclear magnetic resonances were recorded on JEOL JNM-GSX 400 spectrometer. Mass spectra were obtained using GCMSQP 5050 A Shimadzu mass spectrometer, column chromatography and analytical thin layer chromatography was carried out using Merck 77491 and Merck 7730, 60 F-254.

2.2. *Collection and identification*

The materials included fresh and dry fruits of *Ficus palmata* were collected from district Chamoli, Uttarakhand during August-November 2010. These plants were authenticated from Taxonomy Laboratory, Department of Botany, HNB Garhwal University, Srinagar. The voucher specimens GUH 8270 were deposited in the University herbarium for future records.

2.3. *Preparation of plant extract*

The air dried fruits ground to moderately fine powder and soxhlet extracted with petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water using soxhlet apparatus [7]. Each extract was evaporated to dryness under reduce pressure using rotary evaporator. The extracts thus obtained were stored in air tight container at 4°C until further analysis. The hexane fraction was chromatographed on column chromatography eluting with hexane with increase amount of ethyl acetate to give fractions of 200 mL each. Fractions which gave similar spots on the thin layer chromatography (TLC) with appropriate solvent system were combined.

2.4. *Bacterial strains*

The microorganisms (*Bacillus cereus, salmonella entericatyphim, Staphyloccus aureus, staphyloccus epidermidis* and *streptococcus pyogenes)* used in this investigation were obtained from the culture collection & gene bank, institute of microbial technology, Chandigarh, India, (Customer no. 3921).

2.5. *Isolation methods*

The 30.0 gm of the methanolic fraction of the ethanolic extracts of the leaves of *Ficus palmata* were subjected to column chromatography over Merck silica gel PF_{254} (70-230 Mesh ASTM) and eluting with petroleum ether, petroleum ether/chloroform, chloroform and chloroform/methanol to give 50 fractions. Whereas thin layer chromatography was performed on commercially TLC glass sheets precoated with Merck silica gel 60 F-254 (0.2 mm thickness).

2.6. *Antibacterial assay*

The disc diffusion assay methods were used to determine the growth inhibition of bacteria by plant extracts and chemical constituents [8, 9]. Diluted bacterial culture (100 µl) was spread over nutrient agar plates with a sterile glass L-rod. 50µg/ml and 100 µg/mL of the each sample were applied to each filter paper disc (Whatman No. 1, 5 mm diam.) and allowed to dry before being placed on the agar plate. Each sample was tested in triplicate (3 discs/ plate) and the plates were inoculated at 37°C for 24 h. After incubation, the diameter of inhibition zones was measured with a caliper.

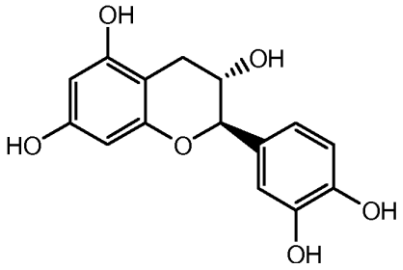
2.7. *Statistical analysis*

Results were expressed as mean (±) standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by multiple

Tukey's comparison tests. A students't'–test value with $p < 0.05$ was considered statistically significant [10].

3. Results and Discussion

The first step towards this goal is the investigated the chemical constituents of the leaves of *Ficus palmata* and their antimicrobial activity of isolated compounds. The results of isolated compounds and their antimicrobial activities are presented in Table 1 and Figs. 2 - 3, respectively.



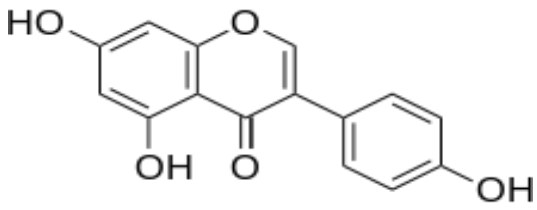
Structure of compound (I), Catechin

State: White powder; m.p. 200-202°C; molecular formula, $C_{15}H_{14}O_6$; molecular weight, 290.27 $gmol^{-1}$.

IR (λ max KBr) cm^{-1} : 3392, 3267, 2936, 1630, 1524, 1472, 1291, 1199, 1053, 959, 822, 675 and 530. R_f 0.74 (chloroform: methanol). IR (KBr, cm^{-1}): 3392, 3267, 2936, 1630, 1524, 1472, 1291, 1199, 1053, 959, 822, 675, 530. [α] 28+54.6 (C, 0.25, MeOH). UV max nm, DMSO (log ε): 300.5 (0.61).

^{1}H-NMR (400 MHz, DMSO) δ: 9.2 (1H, s, 5-OH), 9.0 (1H, 7-OH), 8.8 (2H, 3/-OH & 4/-OH), 6.7 (2H, dd, J=J=8.5 Hz, H-2/& H-6/), 6.5 (1H, d, J=8.5 Hz, H-5/), 5.9 (1H, d, J=1.2 Hz, H-8), 5.7 (1H, d, J=1.2 Hz, H-6), 4.9 91H, br, 3.OH, 4.5 (1H, d, J=7.3 Hz, H-2), 3.8 (1H, m, H-3), 2.7 (1H, m, H-4b), 2.3)1H, m, H-4a).

^{13}C-NMR (100 MHz, DMSO) ppm: 156.3 (C-7), 156.0 (C-5), 155.2 (C-9), 144.7 (C-3/& C-4/), 130.5 (C-1/), 118.3 (C-6/), 115.0 (C-2/), 114.4 (C-5/), 99.0 (C-10), 95.0 (C-8), 93.7 (C-6), 80.9 (C-2), 66.2 (C-3), 27.7 (C-4). MS m/e (% intensity): 290 (M+ 2.4), 267 (0.1), 249 (1.2), 225 (0.8), 207 (2.5), 193 (1.6), 179 (4.0), 167 (4.8), 152 (8.6), 139 (16.5), 123 (12.5), 111 (19.4), 97 (31.9), 71 (48.9), 57 (96.7), 43 (100), 41 (50.6).



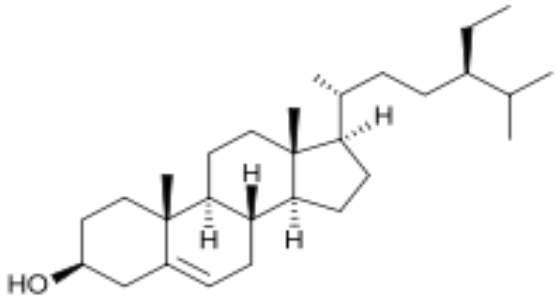
Structure of compound (II), Genistein

State: White powder; m.p. 218-220°C; molecular formula, $C_{15}H_{10}O_5$; molecular weight, 270.24 $gmol^{-1}$.

IR (λ max KBr) cm^{-1}: 3413, 3133, 1652, 1570, 1504, 1476, 1274, 1044, 886, 790, 608 and 516. [α] 28+25.5 (C, 0.85, MeOH). UV max nm, MeOH (log ε): 301.0 (0.25).

^{1}H-NMR (400 MHz, DMSO) δ: 12.9 (1H, s, 5-OH), 8.1 (1H, s, H-2), 7.4 (2H, d, J=3.4 Hz, H-2/& H-6), 6.9 (2H, d, J=3.4 Hz, H-3/& H-5/), 6.4 (1H, d, J=1.7 Hz, H-8/), 6.3 (1H, d, J=1.7 Hz, H-6).

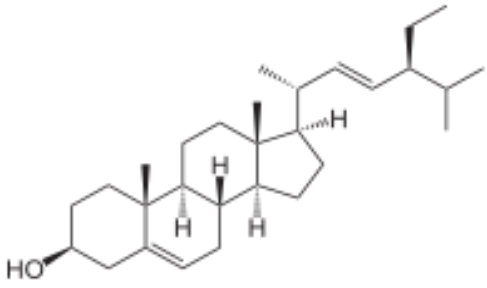
^{13}C-NMR (100 MHz, DMSO) ppm: 180.3 (C-4), 163.5 (C-7), 161.2 (C-4), 157.6 (C-5), 156.2 (C-9), 153.8 (C-2), 130.2 (C-2/& C-6/), 122.2 (C-1/), 121.6 (C-3), 115.2 (C-3/ & C-5/), 104.5 (C-10), 98.9 (C-6), 94.0 (C-8). MS m/e (% intensity): 270 (M+ 33.3), 248 (4.4), 241 (2.2), 217 (3.3), 207 (3.3), 189 (3.3), 173 (4.4), 165 (4.4), 154 (8.9), 153 (61.1), 138 (4.4), 135 (13.3), 124 (61.1), 118 (100), 111 (20.0), 105 (13.3), 96 (30.0), 89 (56.7), 84 (41.1), 77 (26.7), 69 (61.1), 66 (77.8), 63 (35.6), 55 (25.6), 51 (30.0).



Structure of compound (III), β-Sitosterol

State: White powder; m.p. 136-140°C; molecular formula, $C_{29}H_{50}O$; molecular weight, 414.71 $gmol^{-1}$.

β-Sitosterol: 11.87 (C-18), 11.87 (C-29), 18.74 (C-26), 19.02 (C-21), 19.39 (C-19), 19.82 (C-27), 21.07 (C-11), 23.02 (C-28), 24.27 (C-15), 26.06 (C-25), 28.22 (C-16), 29.09 (C-23), 31.46 (C-2), 31.85 (C-7), 31.85 (C-8), 33.90 (C-22), 36.14 (C-20), 36.46 (C-10), 37.21 (C-1), 39.76 (C-12), 42.26 (C-4), 42.26 (C-13), 45.78 (C-24), 50.11 (C-9), 56.02 (C-17), 56.72 (C-14), 71.73 (C-3), 121.68 (C-6), 140.75 (C-5).



Structure of compound (IV), Stigmasterol

State: White powder; m.p. 174-176°C; molecular formula, $C_{29}H_{48}O$; molecular weight, 412.69 g mol^{-1}.

Stigmasterol: 11.97 (C-29), 12.24 (C-18), 19.02 (C-26), 19.39 (C-19), 21.07 (C-11), 21.07 (C-21), 21.20 (C-27), 24.47 (C-15), 25.40 (C-28), 28.93 (C-16), 31.64 (C-2), 31.85 (C-7), 31.85 (C-8), 31.85 (C-25), 36.46 (C-10), 37.21 (C-1), 39.65 (C-12), 40.47 (C-20), 42.26 (C-4), 42.26 (C-13), 50.11 (C-9), 51.19 (C-24), 55.90 (C-17), 56.86 (C-14), 71.73 (C-3), 121.68 (C-6), 129.26 (C-23), 138.31 (C-22), 140.75 (C-5).

The (III) and (IV) components showed absorption band of –OH at 3500-3200 cm^{-1} so it was a steroidal alcohol. ^{1}H-NMR spectrum showed signal of CH-OH at δ 3.52, CH=CH at δ 5.09 and C-CH at δ 5.35. The ^{1}H-NMR spectrum agreed with the ^{13}C-NMR spectrum, which showed the signals of C-OH at δ 71.73 and C=C at δ 121.68, 129.29, 138.31, 140.75. ^{1}H-NMR and ^{13}C-NMR spectra of components (III) and (IV) were identical with 1:1 mixture of β-sitosterol and stigmasterol. TLC of components (III) and (IV) has the same R_f value with that of the authentic sample. GC and co-injection GC of components (III) and (IV) have the same retention times as that of β-sitosterol and stigmasterol and showed the composition of 35% β-sitosterol and 65% of stigmasterol.

R_f 0.47 (silica gel/methanol: chloroform =1: 49); IR λ max (KBr): 3500-3200 (O-H), 3020 (C=CH), 2940, 2860, 1640, 1460, 1380, 1060, 1020, 970, 960, 800 cm^{-1}.
^{1}H-NMR ($CDCl_3$) δ (ppm): 0.68-2.32 (m, C, CH, CH_2, CH_3), 3.52 (b, OH), 5.09 (t, CH=CH), 5.35 (d, =CH), ^{13}C-NMR ($CDCl_3$) δ (ppm):

Four compounds have been successfully isolated from *Ficus palmata* by column chromatography. These compounds have been identified to be Catechin (1), Genistein (2), β-Sitosterol (3) and Stigmasterol (4). Compound (I) was obtained as white powder (10.5 mg) from methanol extract of the leaves of *Ficus palmata* with melting point 200-202°C and molecular weight 290.27 $gmol^{-1}$. Compound II was identified as genistein based on IR, ^{I}H, ^{13}C-NMR, MS and on comparison with published data. β-Sitosterol (III) was isolated as white powder with melting point 136-140°C and molecular weight 414.71 g mol^{-1}. The chemical test and the spectral data obtained for these compounds were in agreement with published data. Stigmasterol (IV) was also obtained from the methanol fraction of the ethanol extract of the leaves of *Ficus palmata* as white powder with melting point 174-176°C. Based on the spectral data and on comparison with literature values the compound was identified as stigmasterol.

The four compounds isolated showed antimicrobial activity towards the *Bacillus cereus, salmonella entericatyphim, Staphyloccus aureus, staphyloccus epidermidis* and *streptococcus pyogenes*. The activity ranged strong based on the diameter of inhibition zones. However, no antifungal activity was observed against the two species of fungi. The antibacterial screening on the compounds showed that Genistein and catechin exhibited the stronger antibacterial activity (Table 1 and Figs. 2 - 3).

Table 1. Antibacterial activity of isolated compounds from the leaves of *Ficus palmata*.

Bacterial Name		Erythromycin	Catechin	Genistein	β-Sitosterol	Stigmasterol
Genus /Species /Subspecies	MTCC (Code)	50 µg /mL	100µg /mL	100µg /mL	100µg /mL	100µg /mL
Bacillus cereus	1272	35	22	23	19	18
Salmonella entericatyphm	98	30	20	21	15	16
Staphyloccus aureus	902	30	21	20	16	17
Staphyloccus epidermidis	435	25	18	19	18	15
Streptococcus pyogenes	1925	30	22	20	16	14

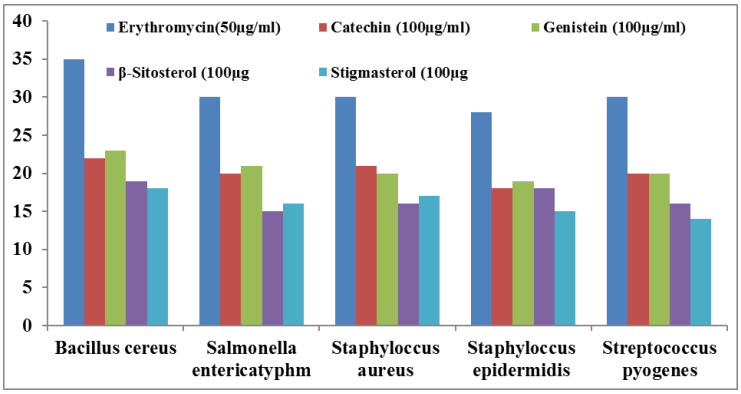


Fig. 2. Zone of inhibition of Catechin, Genistein, β-Sitosterol and Stigmasterol against five bacteria compared with erythromycin.

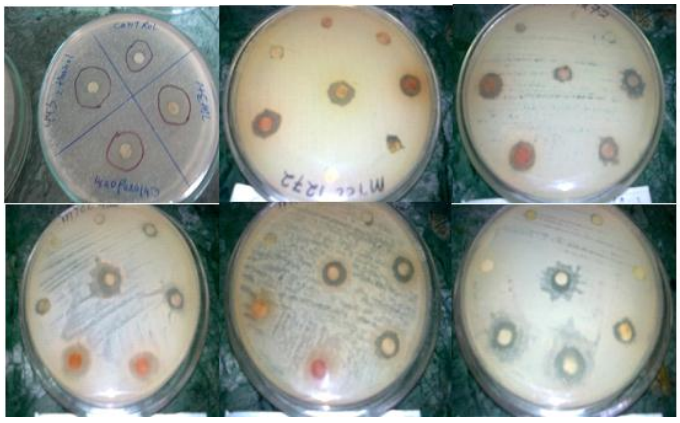


Fig. 3. Zone of inhibition of Catechin, Genistein, β-Sitosterol and Stigmasterol against five bacteria compared with erythromycin.

4. Conclusion

It can be concluded that the methanolic fraction of the leaves of *Ficus palmata* possess potent antimicrobial activity thus validating the ethno pharmacological claims. This is the first time, to report the above compounds present in the methanol extract of *Ficus palmata* leaves. On the basis of our results, *Ficus palmata* appears to have potential for treatment of antimicrobial diseases. It should, however, be explored as a functional medicinal plant for isolating the active ingredients along with animal studies in vivo.

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