

## General Summary

*Ficus platypoda* (Miq.) A. Cunn. known as desert fig or rock fig is endemic to central and northern Australia, and Indonesia. The fruit can be eaten when soft and ripe. Horticulturally, it is suitable for use in bonsai; its tendency to form a wide trunk base and small leaves being attractive features.

*Ficus lyrata* Warb. (*Ficus pandurata* Hance), known as fiddle leaf fig, is indigenous to tropical central and west Africa. It is used as a container or above-ground planter, shade tree and suitable for growing indoors.

**The present study includes the following:**

### **Part I: DNA profiling of the selected species**

The extracted DNA of each of the two *Ficus* species was amplified using eleven decamer primers to detect their genetic variability. The eleven primers of arbitrary sequences generated 130 and 140 fragments in *Ficus platypoda* (Miq.) A. Cunn. and *Ficus lyrata* Warb., respectively.

The eleven primers has produced multiple band profiles with a number of amplified DNA fragments ranging from 16 when OPM-5 and OPB-17 were used in *Ficus lyrata* Warb. On the other hand, the least number of fragments was 8, being produced by OPE-5 in *Ficus lyrata* Warb. and OPA-6 in *Ficus platypoda* (Miq.) A. cunn.

The low percentage of similarity coefficient indicates that the two species are not closely related.

### **Part II: Comparative phytochemical study**

#### **Chapter I: Proximate analysis**

Determination of the pharmacopoeal constants of the leaves of the two species revealed that the total and acid insoluble ashes were higher

in *Ficus lyrata* than *Ficus platypoda*. while water soluble ash and crude fibres contents were close in both species.

**Chapter II:** Preliminary phytochemical screening.

Phytochemical screening of the leaves and stems of the two *Ficus* species revealed the following:

1. Carbohydrate and/or glycosides, hydrolysable and condensed tannins, free and combined flavonoids as well as sterols and/or triterpenes were present in the leaf samples. However, they were detected in traces in the stem samples. Coumarins are found only in traces in the leaves of both species.
2. All the tested samples showed negative response when tested for crystalline sublimate, steam volatile substances, saponins, alkaloids and/or nitrogenous bases, free and combined anthraquinones and cardiac glycosides.

**Chapter III:** Successive extraction and TLC investigation of the extractives.

The air dried powdered leaves of *Ficus Platypoda* (Miq.) A. Cunn. and *Ficus Lyrata* Warb. (2 kg, each) were successively extracted with *n*-hexane, dichloromethane, ethyl acetate and methanol. Percentage yields and organoleptic characters of the different extracts were recorded. The successive extracts were also screened by TLC. This study led to the following conclusions:

1. *n*-Hexane and dichloromethane extracts showed the highest quantities in both leaves; while the more polar extractives *viz.*, ethyl acetate and methanol showed less quantities than the previous non polar extracts.
2. Sterols and/or triterpenoids occur in the *n*-hexane and dichloromethane extractives. On the other hand, phenolics compounds were found in the ethyl acetate extracts.

3. Based on their chromatographic profiles the *n*-hexane and ethyl acetate extracts of the leaves of *Ficus platypoda* and dichloromethane extract of the leaves of *Ficus lyrata* were selected for isolation of their major constituents.

**Chapter IV:** Investigation of the *n*-hexane extracts of *Ficus platypoda* and *Ficus Lyrata*.

**A. GLC analysis of the unsaponifiable and saponifiable lipoidal matters.**

- **Preparation of the lipoidal matters:** The leaves of *Ficus Platypoda* (Miq.) A. Cunn. and *Ficus Lyrata* Warb. (2 kg, each) were successively extracted with *n*-hexane, dichloromethane, ethyl acetate and methanol. The solvent-free residues (*n*-hexane extract) amounted to yield 44.5 and 20 g, respectively.
- **Preparation of the unsaponifiable and saponifiable matters (USM and FA):** The *n*-hexane extract of the leaves of *Ficus Platypoda* and *Ficus Lyrata* (10 g) was separately saponified. The USM and FA were separated. They represent 46 & 74.8% and 20 & 15% of the total lipoidal content, respectively. The fatty acids, in each case, were methylated to yield fatty acid methyl esters (FAME).
- **GLC analysis of the unsaponifiable matters (USM):**
  1. The number of the identified components in the unsaponifiable matter of the leaves of *Ficus platypoda* (15) is higher than that of *Ficus lyrata* (11).
  2. The percentage of the total hydrocarbons was slightly higher in *Ficus lyrata* (59.73%) than that of *Ficus platypoda* (53.77%). *n*-Docosane was the major hydrocarbon in *Ficus platypoda* (21.69%), while *n*-heptacosane was the predominant one in *Ficus lyrata* (33.77%).

3. Sterols and triterpens content was higher in *Ficus lyrata* (26.37%) than that in *Ficus platypoda* (16.9%).  $\beta$ -Sitosterol was the major sterol in both species (5.96% and 4.07% respectively).
4.  $\alpha$  Amyrin (3.01%) was the only detected triterpene. It occurs only in *Ficus platypoda* under the adopted experimental conditions

- **GLC analysis of the fatty acid methyl esters:**

1. The identified components were 8 and 12 representing 97.46% and 84.05 % of the total composition of the fatty acid in the *Ficus lyrata* and *Ficus platypoda*, respectively.
2. The percentage of the total identified saturated and unsaturated fatty acids were higher in *F. lyrata* (97.46, 63.35, and 34.35%) than *F. platypoda* (84.05, 51.5, and 32.55%). Palmitic and carboceric acids were the major saturated fatty acids in *F. platypoda* and *F. lyrata* (22.07 and 35.72% respectively).
3. Linoleic acid was the predominant usaturated fatty acid (18.66 and 16.70%) in both species respectively. Linolenic acid (6.74%) was detected only in *F. Platypoda*.

**B. Isolation of the major constituents of the unsaponifiable matter of the leaves of *Ficus platypoda*.**

The unsaponifiable matter was fractionated using column chromatography. Two steroidal compounds ( $\beta$ -sitosterol and  $\alpha$ -spinasterol) and two triterpenoid ( $\alpha$ -amyrin and  $\beta$ -amyrin) were isolated.

**Chapter V: Investigation of the dichloromethane extract of *Ficus lyrata*.**

The dichloromethane fraction was subjected to VLC using eluents of increasing polarity to give one main fraction. This fraction was purified by successive column chromatography to give two compounds, **C<sub>5</sub>** (quercetin) and **C<sub>6</sub>** ( $\beta$ -sitosterol-O- $\beta$ -D-glucoside). Their structures were identified based on physico-chemical data, spectral data, and comparison with previously published data.

**Chapter VI:** Investigation of the ethyl acetate extract of *Ficus platypoda*

The ethyl acetate extract was fractionated on silica gel column to give four main fraction (fraction A, B, C and D). These fractions were purified by successive column chromatography to give four phenolic compounds [4-(3- acetoxy-2-hydroxypropoxy)-3-hydroxybenzoic acid (**C<sub>7</sub>**), kaempferol 3-O- glucoside (**C<sub>8</sub>**), quercitrin (**C<sub>9</sub>**), and rutin(**C<sub>10</sub>**)]. Their structures were identified based on physico-chemical data, spectral data and comparison with previously published data. Compound **C<sub>7</sub>** was isolated in this study for the first time from nature.

**Chapter VII:** Quantitative estimation of the phenolic content of the ethyl acetate extract of the leaves of *Ficus platypoda*

**It is revealed that;**

- (3- Acetoxy-2-hydroxypropoxy)-3-hydroxybenzoic acid, kaempferol 3-O- glucoside, quercitrin and rutin are the main phenolics present in the *Ficus platypoda*
- Compound **C<sub>7</sub>** [(3- acetoxy-2-hydroxypropoxy)-3-hydroxybenzoic acid] was the major phenolic constituents followed by compound **C<sub>8</sub>** (kaempferol 3-O- glucoside). While compound **C<sub>9</sub>** (quercitrin) and **C<sub>10</sub>** (rutin) occur in a lesser quantities.

**Part III: Biological Studies****1. Acute toxicity study**

Acute toxicity study in mice for the total 80% ethanol extracts of *Ficus platypoda* and *Ficus lyrata* leaves revealed the safety of both extracts up to (2 g/kg b.wt) and showed no signs of toxicity or mortality up to 15 days. Blood samples were obtained, for estimation of blood hemoglobin (Hb), red blood cell count (RBCs) and total leukocytic count (TLC). No behavioral changes were remarked after analysis.

Based on the previous results, the doses used for pharmacological studies were 200 and 400 mg/kg b.wt, which represent 1/10, and 1/5 of

the maximum soluble dose of each extract in water which induced no mortalities in mice.

## 2. Antihyperglycemic activity

Antihyperglycemic activity of 80% ethanol extracts of the leaves of *Ficus platypoda* and *Ficus lyrata* was evaluated in diabetic rats by measuring the level of glucose.

Results revealed that:-

The 80% ethanol extract of the leaves of *F. platypoda* produced exhibited an activity ( $107.9 \pm 5.817$  and  $64.11 \pm 4.358$ ) at the tested doses (200 and 400 mg/kg/day, respectively) higher than the activity of the extract of leaves of *F. lyrata* ( $127.2 \pm 4.359$  and  $127.7 \pm 6.889$ ).

## 3. Antioxidant activity

The results of DPPH assay revealed that the 80% ethanol extract of *Ficus platypoda* was better than *Ficus lyrata* as antioxidant, The  $EC_{50}$  was calculated to be 232.6 and 790.9  $\mu\text{g/ml}$ , respectively.

## 4. Antimicrobial investigation

The 80% ethanol extracts of the two selected species were investigated for their antimicrobial activities against Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis*), Gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* and *Neisseria gonorrhoeae*) as well as fungi strains (*Asperigillus flavus* and *Candida albicans*) using disc agar diffusion method. The result of antimicrobial screening revealed that:

- The 80% ethanol extract of *Ficus platypoda* has a moderate antibacterial activity against all tested strains. While the 80% ethanol extract of *Ficus lyrata* has a moderate antibacterial activity only against *Bacillus subtilis*
- Both extracts showed no antifungal activity against *Asperigillus flavus* or *Candida albicans*.