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Phytochemical and biological screening of the leaves of *Ficus pandurata* Hance. cultivated in Egypt

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Abstract
Ficus is one of medicinally important plant which have many of the biological activities like hypotensive, anti-inflammatory, laxative and anti-rheumatic. In present investigation carried out the phytochemical and biological screening of alcoholic extract of *Ficus pandurata* leaves. The study revealed that the presence of various secondary metabolites such as carbohydrates and /or glycosides, unsaturated sterols, triterpenoids, tannins, flavonoides and these components may have supported the medicinal properties of the plant species. In addition the extracts from leaves showed anti-inflammatory, anti-pyretic and analgesic activities. Furthermore the obtained results may provide support to explore the plant for isolation of the active constituents responsible for these activities.

Keywords: *Ficus pandurata*, anti-inflamatory, anti-pyretic, analgesic, triterpenes and sterols.

1. Introduction
The genus Ficus (Moraceae) is a very large genus of at least 800 species and widely spread in tropical and subtropical countries. Some of Ficus species are cultivated for their edible fruits (*Ficus sycomorus* Linn.), while others for providing shade and as ornamental plants [1-3]. Many phytoconstituents including triterpenes and sterols were isolated and identified from different species of Ficus [4-15]. In folk medicine, Ficus plants are reported to have hypotensive and antidiabetic activities, also it is used to treat cough, chest conditions and also it is used as mild laxative, galactagogue, antirheumatic, digestive and as anthelmintic against intestinal parasites [16-18]. It has been also used as anti-inflammatory in urinary tract, in sore throat, ulcerated nose, to reduce fever, to cure tuberculosis and piles. Externally, they have been to treat postulous, eczema, to cure tinea, for leprosy, to treat cracks in the soles of the feet and dressing to boils [16-18]. Several Ficus species are indigenous to Egypt, such as *Ficus sycomorus* Linn, *Ficus pseudosycomorus* Decene, *Ficus salicifolia* Vahl, other species are recently introduced, such as *Ficus asperrima* Linn, *Ficus benjamina* Linn, *Ficus elastica* Roxb., *Ficus glomerata* Roxb, *Ficus eriobatriods* Linn, *Ficus hisptida* Linn, *Ficus infectoria* Roxb, *Ficus macrophylla* Linn, *Ficus platiboda* Linn, *Ficus pareelli* Veitch, *Ficus religiosa* Linn and *Ficus pandurata* Hance [19-20]. This work describes the phytochemical and biological activites of the extracts from the leaves of *Ficus pandurata* Hance cultivated in Egypt.

2. Material and Methods
2.1 Collection, Identification and preparation of the plant material
The plant material (leaves) was collected in the period from March to April 2013 from gardens of the Faculty of Pharmacy, Assiut University. The plant was kindly identified and authenticated by Prof. Dr. Salah EL-Nagar (Professor of Botany, Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt). The samples were gathered during flowering and fruiting stages. The fresh plant separately air-dried, powdered and then stored in dry sealed glass bottles until use.

2.2 Animals and drugs
Male albino rats (each 100 – 120 g) and mice (each 20 –25 g) were used. Animals were bred and housed under standardized environmental conditions in the Pre-Clinical Animal House, Pharmacology Department, Faculty of Medicine, Assiut University, Assiut, Egypt. They were fed with standard diet with free access to tap water and kept under a 12/12 hours light/dark cycle. Indomethacin was used as standard antipyretic and anti-inflammatory (El-Nile Co., Egypt). Acetyl salicylic acid was used as standard analgesic (The Arab Drugs Co., Egypt) (ADWIC).

2.3 Extraction Procedure
The air-dried, powdered leaves (300 g) were exhaustively extracted with cold maceration method with sufficient quantity of 70% methanol for 7 days at room temperature. The methanolic extract was passed through Whatman No.1 filter paper (Whatman England) and the concentrated extract (30 g). A part of methanolic extract (20 g) was digested with 100 mL distilled water and successively partitioned with *n*-hexane (4 x 400 mL), chloroform (3 x 400 mL), and ethyl acetate (5 x 400 mL). Each fraction was concentrated under reduced pressure to a constant weight to give the corresponding *n*-hexane fraction (6 g), chloroform fraction (3 g), ethyl acetate fraction (2.7 g), and aqueous fraction (7 g) and then freeze dried, stored at 4 ◦C for further investigation.

2.4 Phytochemical screening
The methanolic extract obtained were then subjected to qualitative chemical tests in order to detect the presence of various chemical constituents. Major secondary metabolites classes such as flavonoids, phenol, terpenoids, saponins, tannins, steroids, glycosides, alkaloids and coumarins were screened according to the reference method of [21-23].

2.5 Anti-iflammatory, anti-pyretic and analgesic activities
2.5.1 Preparation of extracts for adminstration
The previously prepared fractions (*n*-hexane, $CHCl_3$, EtOAc and total MeOH extracts) were separately taken in weighed amounts (2, 4 and 8 g) and solubelized in normal saline (0.9%) with the aid of 2% tween 80 to obtain concentrations of 20, 40 and 80 mg/ml. Each fraction was subjected to a preliminary pharmacological screening as anti-inflammatory, analgesic and anti-pyretic activities. A control solution was prepared using the amount of tween 80 in normal saline (placebo).

2.5.2 Acute toxicity
The acute toxicity (LD_{50}) was determined accordining the procedure described by Turner [24]. Mice were divided into five groups, each containing five animals. Different fractions (*n*-hexane, $CHCl_3$, EtOAc and total MeOH extracts) of the leaves of *Ficus pandurata* Hance were administered orally at doses ranging from 1-10 g/kg following standard method. Animals were continuously observed for 2 hrs. to detect changes in the autonomic or behavioral responses and then monitored for any mortality. A Group of animal treated with the vehicle (5% carboxymethyl cellulose sodium served as control.

2.5.3 Anti-inflammatory activity
The anti-inflammatory activity was done according to the method described by Domenjoz *et al* [25] where a pedal inflammation in rat paws was induced by subplantar injection of 20% yeast suspension into the right hind paw of the rats in a dose 1 ml/100 g body weight. Sixty male rats of 100-120 g body weight were divided into ten groups each 6 animals. At the beginning of the experiment, the paws thicknesses were measured in mm using Vernier Caliper. The first group was kept as negative control (non-treated), and injected intraperitoneally by 2% tween 80 in normal saline (solvent mixture), while the second group was injected by indomethacin (reference group) at a dose of 8 mg/kg. The other groups were separately, intraperitoneally administered the different extracts of leaves of *Ficus pandurata* Hance at doses of 200 and 400 mg/kg body weight. After 30 minutes from drug administration, the inflammation was induced by injection of the yeast in the right paw, while the left one was injected by an equal volume of saline solution. The difference between the thicknesses of the two paws was taken as a measure of edema. The anti-inflammatory effect of the tested fractions was estimated by comparing the magnitude of paw swelling in the pretreated animals with those induced in control animals receiving saline. The measurement was carried out at 1, 2, 3, 4 and 5 hrs after injection of the inflammatory agent. The percentage of edema and a percentage of inhibition were calculated as follows: [26]

$$\%\ \text{Variation (edema)} = \frac{(\text{Right paw thickness} - \text{Left paw thickness}) \times 100}{\textit{Right}\ \text{paw thickness}}$$

$$\%\ \text{Inhibition} = \frac{(V_o - V_t) \times 100}{V_o}$$

Where:
V_o: the average paw thickness of control group
V_t: the average paw thickness of the treated group.

2.5.4 Anti-pyretic activity
Ten groups of 6 male rats (100 - 120 g body weight) were used and the rectal temperature was recorded with thermometer. Pyrexia was induced by subcutaneous injection of a 20% (*w/v*) in a volume of 1ml/100g aqueous suspension of yeast [27]. The first group was kept as negative control (non-treated), injected intraperitoneally by 2% tween 80 in normal saline (solvent mixture), while the second group was injected by indomethacin (reference group) at a dose of 8 mg/kg. The other groups were separately, intraperitoneally administered the different extracts of leaves of *Ficus pandurata* Hance at doses of 200 and 400 mg/kg body weight. Rectal temperatures were taken after 1, 2, 3, and 4 hours from administration of tested fractions [27].

2.5.5 Analgesic activity
The method of Koster *et al* was used [28]. Ten groups each of six male albino mice (20-25g) were used. The tested fractions at doses of 200 and 400 mg/kg were orally administered to mice, one hour before interperitoneal injection of 0.6% *v/v* acetic acid, at a dose of 10 ml/kg, while Tween 80 in saline was used as control treatment. Mice in reference group received 150 mg/kg of acetyl salicylic acid (ASA). Writhings that occurred between 5 and 15 minutes after acetic acid were counted.

2.5.6 Statistical Analysis
Data were analysed using the student's t-test and the values were expressed as mean ± S.E. (n= 6 animals).

3. Results and discussion
The present investigation on the preliminary phytochemical screening of 70% methanolic extracts of *Ficus pandurata* Hance leaves summarized in [Table 1]. The 70% methanolic extracts reveals the presence of carbohydrates and/or glycosides, unsaturated sterols, triterpenoides, tannins, flavonoides, and phenolic compound. In addition to traces of volatile substances, coumarines and saponins. alkaloids and/or basic nitrogenous substances, cyanogenic glycosides, cardenolides, anthraquinones and iridoids were not detected. Different phytochemicals have been found to possess awide range of activities, which may help in protection against chronic diseases. Among the great variety of secondry metabolites found in plants, glycosides, flavonoids and tannins have anti-inflammatory activities. Steroids and triterpenoids showed analgesic and central nervous system activities [26, 28-31].

Table 1: Results of phytochemical screening of the leaves of *F. pandurata Hance*.

Plant constituent	Leaves
Carbohydrate and/or glycoside	+
Cardenolides	+
Unsat. Sterols and/or triterpenes	+
Tannins	+
Flavonoids	+
Saponins	±
Phenolic compound	+
Alkaloids and/or basic nitrogenous substances	–
Cyanogenic glycosides	–
Anthraquinones	–
Coumarins	±
Iridoids	–

+ =present ± = traces – = absent

The pharmacological activities of a given plant are associated with the type and nature of secondary metabolites present in them. The need for phytochemical screening has become imperative, since many plants accumulate biologically active substances in various parts and tissues. Phytochemical screening of *Ficus pandurata* Hance leaves revealed the possible presence of phenolics, flavonoids and glycosides. Which have marked pharmacological effects, when administered to man and other animals. The plant has potential source of flavonoids and glycosides would be chance of exploration of anti-inflammatory and antioxidant properties.

Determination of acute toxicity is an important parameter, as the doses used in biological investigation must be lower than the LD50 of different plant extracts. Toxicity of tested extracts from *Ficus pandurata* characterized by irritability, writhing, hypothermia, loss of motor coordination, sedation and deep sleep, followed by death. Toxicity appeared at different dose levels 6, 5, 5, 7 g/kg orally for total MeOH, *n*-hexane, $CHCl_3$ and EtOAc extracts respectively. The present study showed the significant anti-inflammatory, anti-pyretic and analgesic activities of the *n*-hexane, $CHCl_3$, EtOAc and total MeOH extracts of *Ficus pandurata* Hance. The phytochemical constituents of this plant mainly triterpens, sterols and flavonoids contribute to these activities [26, 28-31].

A pretreatment with different extracts as well as total alcoholic extract of *Ficus pandurata* Hance reduced the yeast-induced edema with maximum effects being obtained after 2 hrs. From the previous data it could be concluded that the extracts which showed significant anti-inflammatory activity are *n*-hexane and total MeOH extracts, respectively. They give anti-inflammatory activity at doses 200, 400 mg/kg and the activity continuously for 5 hrs [Tables 2 and 3].

Table 2: Effect of tested extracts of *F. pandurata* Hance on yeast induced paw edema in rats.

Group	Dose mg/kg	Thickness of the right hind paw (mm)				
		1hr	2hrs	3hrs	4hrs	5hrs
Control	-	7.3±0.09	7.4±0.10	7.5±0.09	8.0±0.10	8.3±0.13
Indomethacin	8	5.3±0.07**	4.8±0.08**	4.3±0.06**	4.3±0.03**	4.2±0.06**
Total MeOH	200	5.8±0.15**	5.1±0.06**	5.7±0.12**	6.5±0.09**	6.8±0.17*
	400	5.5±0.18**	4.9±0.08**	5.5±0.10**	6.1±0.08**	6.5±0.11**
n-hexane	200	5.4±0.07**	5.1±0.04**	6.0±0.10**	6.4±0.07**	7.1±0.11**
	400	4.6±0.16**	4.4±0.13**	5.4±0.08**	6.1±0.07**	6.9±0.09**
$CHCl_3$	200	6.3±0.12**	5.6±0.21**	5.7±0.06**	6.1±0.10**	6.9±0.28*
	400	6.0±0.09**	5.3±0.04**	5.5±0.09**	5.9±0.04**	6.4±0.06**
EtOAc	200	6.01±0.09**	5.5±0.09**	5.8±0.13**	6.5±0.12**	7.4±0.09**
	400	5.3±0.14**	5±0.03**	5.6±0.08**	6.3±0.09**	7.1±0.03**

Data are expressed as mean ± S.E, n=6, Differences with respect to the control group were evaluated using the student's t-test (*P< 0.05, **P< 0.01).

Table 3: Inhibitory effects of extracts of *F. pandurata* Hance on yeast induced paw edema in rats.

Groups	Dose mg/kg	Percentage of inhibition				
		1 hr	2 hrs	3 hrs	4 hrs	5 hrs
Control	-	-	-	-	-	-
Indomethacin	8	27.4	35.1	42.6	46.2	49.3
Total MeOH	200	20.5	31.0	24	18.4	18.0
	400	24.6	33.7	26.6	23.4	21.6
n-hexane	200	26.0	31.0	20.0	20.0	14.4
	400	36.9	39.7	28.0	23.4	16.8
$CHCl_3$	200	13.6	22.9	25.3	23.4	16.8
	400	16.7	28.3	26.6	26.2	22.8
EtOAc	200	16.7	25.6	22.6	18.4	10.8
	400	20.5	32.4	25.3	21.0	14.4

The *n*-hexane, $CHCl_3$, EtOAc and total MeOH extracts of *Ficus pandurata* Hance showed significant antipyretic activity at doses 200 and 400 mg/kg. They reduce yeast-induced fever compared with reference compound indomethacin (8 mg/kg) with maximum activity after 2 hrs. The best extracts which give antipyretic activity are *n*-hexane and total MeOH extracts respectively at dose 200 and 400 mg/kg for each, they control the hyperthermia for 4 hour without decrease in activity [Table-4**].**

Table 4: Effect of tested extracts of *Ficus pandurata* Hance on yeast induced pyrexia in rats.

Groups	Dose	Average rectal temperature (°C) ± S.E., n= 6			
	mg/kg	1 hr	2 hrs	3 hrs	4 hrs
Control	-	38.73±0.05	39±0.08	38.93±0.07	38.65±0.18
Indomethacin	8	37.2±0.07**	36.63±0.11**	35.82±0.18**	35.62±0.24**
Total MeOH	200	38.7±0.07	36.85±0.13**	36.33±0.14**	35.85±0.27**
	400	38.37±0.06	36.44±0.22**	36.27±0.3**	35.37±0.25**
n-hexane	200	38.68±0.09	37.47±0.21**	36.47±0.21**	35.2±0.11**
	400	38.42±0.06	36.9±0.21**	35. 97±0.29**	35.13±0.09**
$CHCl_3$	200	38.42±0.10	38.0±0.06*	37.02±0.05**	35.93±0.13**
	400	38.35±0.13	37.1±0.16*	36.3±0.25**	35.33±0.08**
EtOAc	200	38.78±0.06	37.6±0.18**	37.3±0.09**	37.23±0.08**
	400	38.4±0.10	36.9±0.10**	36.82±0.13**	36.78±0.17**

Data are expressed as mean ± S.E, n=6, Differences with respect to the control group were evaluated using the student's t-test (*P< 0.05, **P< 0.01).

The results of analgesic activity of *n*-hexane, $CHCl_3$, EtOAc and total MeOH extracts of *Ficus pandurata* Hance was presented in [Table- 5] and showed that the non-polar fraction (*n*-hexane extract) significantly reduced the number of acetic acid induced writhing, compared with control at a dose of 400 mg/kg. It exhibited an analgesic effect to the same degree as 150 mg/kg acetyl salicylic acid (ASA). The ethyl acetate and total alcoholic extracts have intermediate potency while, the lowest potent extract is the $CHCl_3$ fraction when compared with the other extract.

The plant species studied here can be used as a potential source of useful drugs. It also justifies the folklore medicinal use and the claims about the therapeutic values of this plant as a curative agent. Therefore, further study needed for isolation, identification, purification, characterization, and structural elucidation of bioactive compounds of *Ficus pandurata* Hance that wound be obtained with pharmacological and clinical trials, the compounds leads a promising therapeutic agent.

Table 5: Results of analgesic activity of test extracts of leaves of *Ficus pandurata* Hance.

Group	Dose mg/kg	Writhing response in mice (mean±S.E.), n=6		
		5 min.	10 min.	15 min.
Control	-	43.17±0.47	40±0.57	38.67±0.55
ASA	150	17.17±0.6**	15±0.57**	13.3±0.42**
Total MeOH	200	33.3±0.88**	30.3±0.66**	29.67±0.88**
	400	29.0±0.73**	28.67±0.49**	26.17±0.47**
n-hexane	200	28.0±0.60**	27.0±0.57**	25.67±0.42**
	400	19.7±0.84**	17.67±0.66**	15.67±0.66**
$CHCl_3$	200	31.0±0.57**	28.17±0. 6**	26.17±0.21**
	400	27.2±0.6**	25.5±0.42**	20.83±0.47**
EtOAc	200	30.17±0.6**	29.83±0.47**	25.0±0.57**
	400	27.0±0.73**	25.83±0.47**	22.83±0.47**

Data are expressed as mean ± S.E, n=6, Differences with respect to the control group were evaluated using the student's t-test (*P< 0.05, **P< 0.01).

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