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Research Article

Studies on Ficus Racemosa and Kalanchoe Pinnata

Toxicity Studies and Phytochemical Screening of *Ficus Racemosa* and *Kalanchoe Pinnata*

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Abstract: *Kalanchoe pinnata* and *Ficus racemosa* are naturally occurring plant products that are gaining importance because of their excellent medicinal properties. Determining the toxicity of a plant extract is crucial, based on which the therapeutic dose of extracts can be adjusted. The present study evaluates phytochemical screening and toxicity studies (acute and sub-acute) of the *Kalanchoe pinnata* and *Ficus racemosa* plant products. Phytochemical screening showed the presence of alkaloids, tannins, flavonoids, steroids, phenols, and glycosides. The acute toxicity study of both the plant extracts was performed in Wistar albino rats at a single dose of 2000mg/kg body weight in both genders. Sub-acute toxicity study was performed in Wistar albino rats (both male and female) for 28 days at various doses of 500, 1000, and 2000 mg/kg body weight. The evaluation results of the acute toxicity study did not show any signs of toxicity, behavioral changes, mortality, or differences in gross histopathology appearance. The results of the sub-acute toxicity study also showed no changes in body weight, toxic signs, mortality, or behavioral changes. All biochemical and hematological parameters were in normal ranges comparable to the control group's. At the end of the treatment period, all the rats were sacrificed, and the liver and kidneys were sent for histopathological examination. No abnormalities were observed in the treatment groups. The present study concludes that both plant extracts are safe, and the LD_{50} of both plant extracts was above 2000mg/kg bw.

Keywords: Acute toxicity, sub-acute toxicity, Wistar albino rats, *Kalanchoe pinnata*, *Ficus racemosa*

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1. INTRODUCTION

Plants are a natural source of many drugs which are readily available, safe, and cheap; even today, in developing countries, people still depend on herbal medicines and traditional practitioners. Phytochemical constituents present in different parts of plants are mainly responsible for their biological activity. Extensive research is going on *Ficus racemosa* and *Kalanchoe pinnata* plants[1]. In previous centuries medicinal plants have been known to be the richest source for extracting various therapeutic agents to treat various diseases[2]. Recently different categories and regulatory frameworks at regional and national levels describe medicinal plants as one of the mainstream treatments or as alternate or complementary medicines[3]. The plant ficusracemosa as a whole plant has many therapeutic uses in traditional medicine. It is one of the few plants cited previously in Vedic literature. In addition, it has an important sacred role in yagnas and homas[4]. This plant belongs to the Moraceae family, popularly known as cluster fig, gular or Indian fig tree. The chemical constituents in the leaves, fruits, and roots are responsible for their good medicinal value. This herb is also mentioned in the ancient scriptures of Ayurveda, Siddha, Unani, and homeopathy for its medicinal value in treating diabetes, liver disorders, diarrhea, respiratory and urinary diseases, and haemorrhoids[5]. It is found to be grown in forests and hills all over India, specially cultivated around water streams. Previous studies showed the ethanolic extract of leaves of ficusracemosa showed the presence of saponins, tannins, steroid terpenoids, glycosides, resins, and anthocyanins which may be responsible for its medicinal properties [6]. *Kalanchoe Pinnata*, also known as *Bryophyllum pinnatum*, belongs to the Crassulaceae family. It is called a wonder leaf or mother of thousands as new plantlets arise from the margin of the leaves, which can be cut and cultivated separately[7]. The plant has simple opposite compound leaves. Previous studies showed that it is a good source of anti-cancer treatment[8]. A phytochemical review of this plant study showed the presence of flavonoids, coumarin, sterols, and anthrocyanins[9]. It is a perennial plant that can store water giving the leaves a thick, green, fleshy, and scalloped appearance. It is mostly found in the temperate and tropical regions of Africa, Australia, and America. It is of pharmaceutical importance due to its effects in treating different ailments. The chemical constituents of these herbs are grouped as bufadienolides which have similar structures to that of Digoxin and Digitoxin[10]. Previous studies showed that this plant possesses various pharmacological properties like antihypertensive, hepatoprotective, anti-diabetic, and wound healing activity anti-tumour activity[11]. Another study revealed that phytochemical screening of chloroform extract contains steroids, saponins, glycosides, alkaloids, flavonoids, carbohydrates, and proteins, and petroleum ether extract contains alkaloids and steroids[12]. More clinical trials of these plants are to be conducted to confirm the various pharmacological activities of these plant extracts. Recently, diseases like diabetes and cancer are becoming increasingly common in people of all ages and regions. Lifestyle changes are attributed as the primary reason for the spread of these diseases. Most modern medicines used for treating these diseases are reported to lead to various side effects[13]. In light of this, the search is on for tracing natural medicines that could be extracted from safe and effective plants. By their availability in abundance, multiple usages of all their components, references from ancient literature such as Vedic scriptures, and shreds of evidence found for their richness in the phytochemical activity of *Ficus racemosa* and *Kalanchoe pinnata* appear to be potential sources for extracting natural drugs[14,15]. Although natural drugs are found to be safe, their toxicity is to be evaluated to confirm the lethal dose of the plant extracts. The aim and objective of the present study are to evaluate various phytochemical constituents of hydroalcoholic extract of both plants *Kalanchoe pinnata* and *Ficus racemosa*. Further acute and subacute toxicity studies of both plants are also conducted.

2. MATERIALS AND METHODS

2.1. Plant authentication and Voucher number

All the plants were collected from the Hyderabad district, identified and authenticated by Dr.K.Madhava Chetty, Assistant professor, Department of Botany S.V University Tirupati, with voucher numbers 0811 and 0414 for *Ficus racemosa* and *Kalanchoe pinnata*, respectively, Plant Extraction The freshly collected leaves of *Ficus racemosa* and *Kalanchoe Pinnata* were shade dried and coarsely powdered. The powder was passed through sieve no.40. The sieved powder was stored in an airtight container for further use. Initially, 100g of each dried plant material powder was macerated with hydro alcohol 60% v/v ethanol for 7 days[16]. It was then filtered, the solvent evaporated, and the percentage yield was calculated (Table I) and stored in desiccators until further use.

Table I: Percentage yield of plants *Ficus racemosa* and *Kalanchoe pinnata*

Plant Material	Solvent used	Percentage yield	Nature
Ficus racemosa (FR)	Hydro-alcohol (60:40)	5.5% w/w	semisolid
Kalanchoe Pinnata (KP)	Hydro-alcohol (60:40)	3.9% w/w	semisolid

Table I depicts the yield obtained from kalanchoepinnata leaf and *Ficus racemosa* fruit extract. The percentage yield obtained from the hydroalcoholic extract of fruits of *Ficus racemosa* is remarkably greater than that obtained from the hydroalcoholic extract of leaves of *Kalanchoe pinnata* extract.

2.2. Phytochemical screening

The hydroalcoholic extracts of KP and FR are tested for the presence of different active phytochemicals, including alkaloids, carbohydrates, proteins, steroids, polyphenols, flavonoids, tannins, alkaloids, terpenoids, steroids, phenols, gums and mucilage, glycosides, saponins, terpenes, tannins and flavonoids using the method described by Erum Iqbal et.al[17]. Table II provides details of the phytochemical screening of hydroalcoholic extracts of the leaves of *Kalanchoe pinnata* and fruits of *Ficus racemosa.*

2.3. Materials and methods

All animal studies were performed according to OECD guidelines for the testing of animals "Acute oral toxicity study" guideline no.423 and is approved by the Institutional Animal ethical committee KAMSRC Hyderabad CPCSEA Registration No: 2072/PO/Re/S/19/CPCSEA. (ethical project number KAMSRC/Pharm/IAEC/2020/1.) The sub-acute toxicity studies were conducted according to the OECD guideline 407. The animals were examined and acclimatized

to the new environmental conditions before the start of the experiment.

2.4. *Animals and Their Maintenance*

Albino rats weighing around 150-190g were randomly selected and were maintained at 22 ± 3° C with 30 - 70 % relative humidity. Animals were kept at 12 hours of light & dark cycle. Standard pellet feed and water were provided *ad libitum* to the experimental animals except stated otherwise.

2.5. *Experimental Procedure*

2.6. *Acute toxicity studies*

Rats fasted overnight before dosing. Two female rats were orally administered 2000 mg/kg bw of FR& KP using stainless steel feeding gavage. The rats survived; therefore, two additional rats were administered with 2000 mg/kg b.w, 48-h after dosing the first animal. Since both additional rats survived, two were administered 2000 mg/kg bw 48-h after dosing the previous two animals. Then all 6 animals were observed for signs of abnormality or mortality for 14 days, at least once daily, without further dosing of animals. The body weights were recorded on 1st, 2nd, 4th, 6th, 8th, 10th, 12th and 14thdays. All Six rats were sacrificed after a 14-day observation and were subjected to necropsy. All external and internal lesions (if any) were carefully observed and recorded. As no toxic lesions occurred, no tissue samples were taken for histopathological examination.

2.7. *Sub-acute toxicity studies*

Animals were divided into 7 groups (4-FR and 3-KP) of 10 animals (5 males and 5 females) each. The first group (FR-1 and KP-1) of animals was considered control and administered with saline. The animals' second group (FR-2 and KP-2) were administered with low doses, i.e., KP 500mg/Kg and FR 500mg/Kg, respectively. The third group of animals (FR-3 and KP-3) received a medium dose, i.e., 1000mg/Kg of FR and KP. The fourth group of animals (FR-4 and KP-4) were administered high doses, i.e., 2000mg/Kg of the individual drugs. The drugs were administered seven days a week for 28 days with the help of oral gavage. After 28 days, the control and test group animals (7 groups) were sacrificed. The animals were observed for their appearance, behavior, and toxic signs every day throughout the study. Before the terminal sacrifice of the animals, blood samples were collected by retro-orbital sinus, placed in vacuoles containing EDTA, and evaluated for hematological (table V1, VII) and biochemical parameters (table VIII, IX). All the major organs like kidneys, liver, lungs, heart, brain, pancreas, and stomach) were removed, collected, and preserved in 10% formalin solution for organ weights (table X, XI) and histopathological studies.

2.8. *Statistical Analysis*

In the toxicity study, all the values are expressed as mean ± S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by dun nets test using Graph pad prism 6. $p<0.05$ was considered significant.

3. RESULTS

The crude hydroalcoholic extracts of *Ficus racemosa* and *Kalanchoe Pinnata* were tested for different phytochemicals. The qualitative results are expressed as (+) for their presence and (–) for their absence.

Table II: Phytochemical screening of hydroalcoholic extracts of leaves of *Ficus racemosa* and *Kalanchoe pinnata*

Test	Hydroalcoholic extract of *Ficusracemosa*	Hydroalcoholic extract Of *KalanchoePinnata*
Alkaloids	+	+
Carbohydrates	+	+
Tannins	+	+
Flavonoids	+	+
Steroids &Terpenoids	+	+
Glycosides	++	+
Saponins	+	+
Phenols	+	+
Proteins	-	-
Fixed oils & Fats	-	+

Legends: [" ++" = Moderate reaction after adding of reagent immediately; "+" = low reaction after 10 minutes of adding reagent "–" = absence]

Table III: Frequency of selected ten phytochemicals screened in *Kalanchoe Pinnata leaves and Ficus racemosa* fruits extracts

Qualitative Features	*KalanchoePinnata* leaf extract	*Ficusracemosa* fruit extract
Highest (+++)	0	0
Medium (++)	0	1 (10%)
Lowest (+)	9 (90%)	7 (70%)
Absence (-)	1 (10%)	2 (20%)
Total (N%)	10 (100%)	10 (100%)

In the acute toxicity study, no mortality or clinical signs of toxicity were observed at the dose of 2000mg/Kg of FR and KP extracts. Individual body weights were recorded on 1st, 2nd, 4th, 6th, 8th, 10th, 12th and 14th days following oral administration. The gain in body weight was normal in all the rats. At the end of the study, the animals were sacrificed. No abnormality was seen in any of the organs. Based on these findings, the LD50 of FC and KP is considered to be > 2000mg/Kg per OECD guidelines. Sub-acute toxicity study: The study was done according to the OECD guidelines, as discussed in the experimental procedure. There were no toxic signs and symptoms; no abnormalities were observed in

any of the small doses (500mg/Kg), medium doses (1000mg/Kg), and high doses (2000mg/Kg) when compared to the respective control groups. The body weights of all the animals were recorded weekly (for four weeks). Body weights of all the treatment groups were comparable to that of the control group (table IV, V). Feed consumption was also comparable to the respective control groups.

Table IV: Body weights of animals treated with hydroalcoholic extract of fruits of FR. All the values are expressed as mean ± S.E.M

Gender	Dosage of extract of FC	0 week	1st week	2ndweek	3rd week	4th week
FEMALE	Control	142.83 ± 0.60	150.23±0.66	155.17±6.09	160.83±4.88	164.20±3.35
	500mg/kgbw	144.77±2.09	151.87±3.04	159.00±2.99	169.03±3.38	175.45±2.65
	1000mg/kgbw	140.30±3.08	151.00±3.75	157.13±1.39	163.40±5.02	168.33±3.09
	2000mg/kgbw	141.63±2.30	152.00±2.66	157.47±1.14	160.07±3.57	164.00±3.15
MALE	Control	173.47±1.45	179.23±1.07	180.17±10.31	185.83±7.63	188.07±0.81
	500mg/kgbw	164.33±8.23	171.93±4.12	179.90±6.45	182.70±5.88	190.87±2.96
	1000mg/kgbw	169.23±5.01	173.90±4.07	181.07±2.63	185.90±2.94	189.97±3.76
	2000mg/kgbw	170.23±5.01	183.23±2.57	188.40±3.71	190.57±2.42	193.97±3.88

Table IV shows no marked abnormalities in body weights of control and drug-treated groups, which indicates that the rise in body weights in all groups treated with different doses of hydroalcoholic extracts of *Ficus racemosa* fruits is within the normal range as seen in the control group.

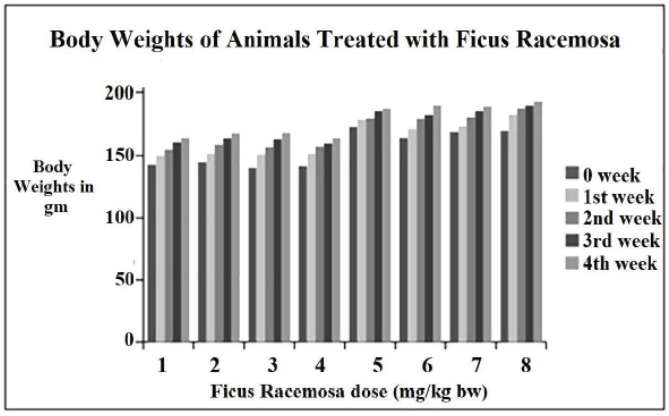

Fig 1: Body weights of animals treated with hydroalcoholic extract of fruits of FR

Table V: Body weights of animals treated with hydroalcoholic extract of leaves of KP. All the values are expressed as mean ± S.E.M

Gender	Dosage of extract of KP	0 week	1st week	2ndweek	3rd week	4th week
FEMALE	Control	142.83 ± 0.60	150.23±0.66	155.17±6.09	160.83±4.88	164.20±3.35
	500mg/kgbw	146.47±2.02	152.53±2.39	159.50±2.63	163.80±1.51	169.73±2.94
	1000mg/kgbw	141.50±3.31	151.00±3.75	157.40±1.55	163.40±5.02	168.23±1.24
	2000mg/kgbw	142.73±2.59	152.00±2.66	158.47±1.20	164.07±4.91	169.07±4.41
MALE	Control	173.47±1.45	179.23±1.07	180.17±10.31	185.83±7.63	188.07±0.81
	500mg/kgbw	165.17±8.33	169.27±6.78	172.60±3.02	183.33±7.11	189.07±6.32
	1000mg/kgbw	170.47±4.80	182.90±2.90	184.37±3.08	186.27±3.56	190.23±5.78
	2000mg/kgbw	171.93±4.65	183.77±2.05	188.70±6.40	192.60±4.15	195.20±2.85

Table V shows no marked abnormalities in body weights of control and drug-treated groups, indicating that the rise in body weights in all groups treated with different doses of hydroalcoholic extracts of *Kalanchoe pinnata* leaves is within the normal range as seen in the control group.

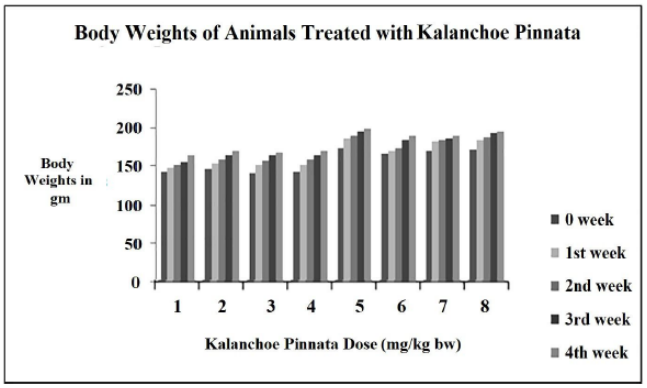

Fig 2: Body weights of animals treated with hydro-alcoholic extract of leaves of KP

Table VI: Haematological parameters of animals treated with hydroalcoholic extract of fruits of FR.

Parameter	FEMALE				MALE			
	CONTROL	500 mg/kgbw	1000 mg/kgbw	2000 mg/kgbw	CONTROL	500 mg/kgbw	1000 mg/kgbw	2000 mg/kgbw
Haemoglobin gm%	16.60 ±0.50	15.60±1.33	15.47±0.52	14.87±0.43	15.07±0.24	15.47±0.26	15.60±0.23	15.33±0.67
RBC million/mm^3	8.87±0.33	8.73±0.63	8.13±0.17	7.80±0.2	7.67±0.13	7.80±0.34	8.40±0.23	7.93±0.17
WBC /mm^3	16600.00±1814.75	15133.33±1524.61	17000.00±3407.83	16000.00±4277.07	11800.00±1039.23	11800.00±1803.27	19400.00±400.82*	14200.00±642.91
Platelets lakhs/mm^3	5.07±0.17	6.93±0.35	6.27±0.06	9.73±2.66	7.00±0.34	7.53±0.54	6.73±0.17	5.73±0.40
MCHC	36.33±0.97	34.97±0.32	36.40±0.26	36.70±0.63	35.23±0.36	36.07±1.19	34.43±0.61	36.03±0.49
MCH	24.87±0.17	24.53±0.43	25.23±0.46	23.97±0.40	25.27±0.27	24.87±0.40	23.90±0.41	24.10±0.43
MPV	6.50±0.17	6.47±0.20	6.80±0.05	6.70±0.1	6.43±0.18	6.77±0.03	6.67±0.14	6.60±0.17
MCV	51.13±0.40	52.77±0.26	52.07+0.14	53.00+0.55	52.60+0.15	53.37+0.38	52.77+0.33	53.60+0.45
RDW	14.33+0.14	14.33+0.08	14.63+0.03	14.33±0.08	14.37±0.21	14.73±0.03	14.77±0.03	14.70±0.1
Neutrophils	3.00±0.57	3.67±0.66	2.33±1.34	4.67±2.69	2.00±0.57	1.67±0.66	4.33±0.33	2.33±0.33
Lymphocytes	90.33±1.20	89.00±0.57	93.33±1.76	88.67±1.76	91.67±2.02	93.33±1.76	89.33±0.66	93.33±0.66
Eosinophils	4.00±0.57	5.00±0.00	2.67±1.20	4.33±0.66	4.00±1.15	3.33±0.88	4.33±0.33	2.33±0.33
Monocytes	2.67±0.33	2.33±0.33	1.67±0.33	2.33±0.33	2.33±0.33	1.67±0.33	2.00±0.33	2.00±0.00
Basophils	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Abnormal cells	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Haemoparasites	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL

All the values are expressed as mean ± SEM (n=5)

Table VI: Establishes all the hematological parameter values are normally compared to the control group; NO statistical significance is noted in all the groups treated with *Ficus racemosa* fruit extracts compared to the control group.

Table VII: Haematological parameters of animals treated with hydroalcoholic extract of leaves of KP

Parameter	FEMALE				MALE			
	CONTROL	500 mg/kgbw	1000 mg/kgbw	2000mg/kgbw	CONTROL	500 mg/kgbw	1000 mg/kgbw	2000mg/kgbw
Haemoglobin gm%	16.60 ±0.50	15.60±0.30	17.13±0.94	17.00±1.77	15.07±0.24	15.23±0.21	15.33±0.37	15.87±0.52
RBC million/mm^3	8.87±0.33	8.87±0.33	9.13±0.73	9.13±0.92	7.67±0.13	7.67±0.13	8.20±0.2	7.60±0.64
WBC /mm^3	16600.00±1814.75	16933.33±1484.73	9333.33±1297.86*	10866.67±1443.76*	11800.00±1039.23	11800.33±1039.23	12466.67±1271.91	7933.33±2545.80**
Platelets lakhs/mm^3	5.07±0.17	5.13±0.20	4.67±0.24	6.53±1.50	7.00±0.34	7.10±0.32	7.33±0.06	7.47±0.54
MCHC	36.33±0.97	34.47±0.20	35.90±0.11	35.73±0.52	35.23±0.36	33.17±0.60	34.20±0.80	36.03±0.49
MCH	24.87±0.17	24.07±0.26	24.80±0.28	23.83±0.53	25.27±0.27	23.80±0.40	24.93±0.40	23.83±0.53
MPV	6.50±0.17	6.87±0.06	6.87±0.12	6.83±0.03	6.43±0.18	6.57±0.12	6.87±0.12	6.73±0.06
MCV	51.13±0.40	52.57±0.39	52.10±0.17	53.00±0.55	52.60+0.15	52.57±0.14	52.33±0.31	53.00±0.55

RDW	14.33+0.14	14.33±0.08	14.37±0.23	14.13±0.23	14.37±0.21	14.03±0.21	14.57±0.08	14.20±0.17
Neutrophils	3.00±0.57	2.33±0.88	4.33±0.88	3.33±1.85	2.00±0.57	5.00±2.30	5.33±1.45	1.00±0.00
Lymphocytes	90.33±1.20	91.33±1.45	87.33±2.40	90.33±3.17	91.67±2.02	88.67±4.09	89.33±0.33	95.00±0.57
Eosinophils	4.00±0.57	3.67±0.33	5.00±1.00	4.00±1.00	4.00±1.15	3.67±1.20	3.33±0.66	2.33±0.33
Monocytes	2.67±0.33	2.67±0.33	3.33±0.66	2.33±0.33	2.33±0.33	2.67±0.66	2.00±0.57	1.67±0.33
Basophils	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Abnormal cells	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Haemoparasites	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL

All the values are expressed as mean ± SEM (n=5) * P> 0.005, ** P> 0.001

Table VII establishes that all the hematological parameters in drug-treated groups are in the normal range as a control group. A slight increase in WBC count was observed in groups treated with *Kalanchoe pinnata* leaves at doses 500mg/kgbw and 1000mg/kg BW which may be coincidental and not have any toxicological importance.

Table VIII: Biochemical parameters of animals treated with hydroalcoholic extract of fruits of FR.

	Biochemical parameters												
	Dosage of Ficus racemosa	**Proteing /dl**	**Albuming /dl**	**Globuling /dl**	**AB:GB Ratio**	**Urea mg/dl**	**Creatinine mg/dl**	**Uric acid mg/dl**	**Cholesterol mg/dl**	**Triglycerides mg/dl**	**HDL mg/dl**	**LDL mg/dl**	**VLDL mg/dl**
FEMALE	control	7.10±0.17	4.00±0.2	2.40±0.05	1.67±0.05	59.67±6.88	0.83±0.03	3.50±0.32	92.33±4.91	86.33±2.60	54.67±18.18	20.43±8.32	17.00±0.57
	500mg/kgbw	8.10±0.72	3.20±3.14	2.10±0.05	1.53±0.04	47.33±4.05	0.80±0.03	3.47±0.27	84.00±6.65*	85.00±19.85	47.67±10.86	20.33±8.33	17.00±4.40
	1000mg/kgbw	8.17±0.23	3.47±0.23	2.33±0.03	1.49±0.10	47.00±3.21	0.87±0.03	3.20±0.05	85.00±2.08*	87.67±18.12	55.00±3.51	12.33±5.78	17.67±3.71
	2000mg/kgbw	7.73±0.26	2.73±0.47	2.43±0.12	1.14±0.23	49.33±4.05	0.80±0.06	3.47±0.26	76.00±3.51*	56.00±2.51**	40.33±8.19	24.87±9.66	11.00±0.57
MALE	control	10.07±0.08	3.43±0.08	2.20±0.05	1.56±0.03	54.67±2.60	0.73±0.03	3.37±0.35	93.33±6.66	84.33±8.51	60.00±3.60	16.02±2..32	17.00±1.52
	500mg/kgbw	10.50±0.15	3.27±0.08	2.27±0.08	1.45±0.06	63.33±3.28	0.73±0.03	3.60±0.2	93.33±3.17	67.33±1.20*	60.33±8.21	19.66±3.33	13.33±0.33
	1000mg/kgbw	8.30±1.80*	3.13±0.12	2.20±0.05	1.43±0.08	44.67±1.33	0.72±0.02	3.27±0.03	71.00+2.08**	65.00±4.00*	44.67±5.20	13.67±3.28	12.67±0.66
	2000mg/kgbw	7.63±0.35	3.50±0.23	2.10±0.05	1.68±0.15	46.00±3.51	0.80±0.05	3.37±0.18	75.00±7.09**	63.00±12.16**	39.00±6.42	23.33±1.45	12.67±2.33

All the values are expressed as mean ± S.E.M (n = 5) * P> 0.005, ** P> 0.001

Table VIII: Reflects that all the biochemical parameters in the control and drug-treated groups were in the normal range, whereas a decrease in cholesterol and Triglyceride levels was observed when compared to the control group in different drug-treated groups.

Table IX: Biochemical parameters of animals treated with hydroalcoholic extract of leaves of KP.

	Biochemical parameters											
	Dosage of KP	Proteing/ dl	Albuming /dl	Globuling /dl	AB: GB Ratio	Urea mg/dl	Creatinine mg/dl	Uric acid mg/dl	Cholesterol mg/dl	Triglycerides mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
FEMALE	control	7.10±0.17	4.00±0.2	2.40±0.05	1.67±0.05	59.67±6.88	0.83±0.03	3.50±0.32	92.33±4.91	86.33±2.60	54.67±18.18	20.43±8.32	17.00±0.57
	500mg/kgbw	10.27±0.43	3.77±0.21	6.50±0.45	0.53±0.03	46.33±6.38	0.77±0.03	3.47±0.03	92.33±4.91	87.00±2.64	64.67±18.18	16.00±8.32	17.00±0.57
	1000mg/kgbw	8.60±0.76	3.57±0.24	5.03±0.52	0.63±0.03	53.00±7.21	0.80±0.05	3.63±0.14	87.33±5.69	84.67±13.66	38.00±2.30	32.67±7.51	16.67±2.66
	2000mg/kgbw	10.50±0.26	3.03±0.03**	7.47±0.29	0.33±0.03**	45.00±1.52	0.83±0.01	3.60±0.25	90.67±1.76	100.67±4.99	45.00±4.04	25.33±4.66	20.33±0.88
MALE	control	10.07±0.08	3.43±0.08	2.20±0.05	1.56±0.03	54.67±2.60	0.73±0.03	3.37±0.35	93.33±6.66	84.33±8.51	60.00±3.60	16.02±2..32	17.00±1.52
	500mg/kgbw	10.37±0.12	3.70±0.15	6.57±0.12	0.53±0.03	66.00±5.13	0.77±0.03	2.93±0.14	83.33±6.66	84.33±8.51	75.00±3.60	11.67±5.20	17.67±2.65
	1000mg/kgbw	9.70±0.3	3.53±0.06	5.50±1.00	0.53±0.03	53.33±4.25	0.83±0.03	3.60±0.05	74.67±3.75**	78.33±16.04*	47.33±2.90	11.67±5.20	15.67±3.17
	2000mg/kgbw	9.73±0.23	3.17±0.08	6.50±0.28	0.43±0.03	65.33±6.38	0.73±0.03	3.33±0.14	81.67±2.84	75.67±8.41*	48.33±7.12	18.33±8.45	15.00±1.73

All the values are expressed as mean ± S.E.M (n = 5) * P> 0.005, ** P> 0.001

Table IX Reflects that all the biochemical parameters in the control and drug-treated groups were in the normal range. Still, there is a decrease in albumin and AB: GB ratio values at 2000mg/kg bw in females and a decrease in cholesterol levels.

Table X: Organ weights of animals treated with hydroalcoholic extract of fruits of FR. All the values are expressed as mean ± S.E.M.

Gender	Dosage of FC	Liver	Right kidney	Left kidney	Right kidney measurements	Left kidney measurements
FEMALE	Control	5.10±0.56	1.13±0.05	0.57±0.03	1.6*1.1	1.5*0.8
	500mg/kgbw	4.18±0.19	1.0±0.01	0.49±0.01	1.1*0.8	1.6*1.1
	1000mg/kgbw	5.75±0.27	1.36±0.07	0.66±0.04	1.6*1.4	1.3*1.1
	2000mg/kgbw	4.96±0.48	1.06±0.04	0.52±0.02	1.5*0.9	1.5*0.6
MALE	Control	7.99±0.79	1.62±0.09	0.81±0.04	1.5*0.9	1.6*1.1
	500mg/kgbw	9.66±0.83	1.99±0.08	1.03±0.05	1.6*0.9	1.6*1.1
	1000mg/kgbw	7.96±1.02	1.63±0.16	0.84±0.08	1.5*0.6	1.6*0.6
	2000mg/kgbw	9.13±2.18	1.69±0.17	0.85±0.09	1.5*0.9	1.8*1.1

Table X depicts that all organ weights are normal range in both the sexes in drug-treated groups compared to control group weights.

Table XI: Organ weights of animals treated with hydroalcoholic extract of leaves of KP. All the values are expressed as mean ± S.E.M.

Gender	Dosage of KP	Liver	Right kidney	Left kidney	Right kidney measurements	Left kidney measurements
FEMALE	Control	5.10±0.56	1.13±0.05	0.57±0.03	1.6*1.1	1.5*0.8
	500mg/kgbw	5.47±0.4	1.17±0.06	0.59±0.03	1.3*0.7	1.6*0.8
	1000mg/kgbw	5.30±1.1	1.27±0.13	0.63±0.05	1.5*1.1	1.6*0.8
	2000mg/kgbw	4.6±0.5	1.07±0.06	0.53±0.02	1.5*0.9	1.4*0.8
MALE	Control	7.99±0.79	1.62±0.09	0.81±0.04	1.5*0.9	1.6*1.1
	500mg/kgbw	6.94±0.96	1.58±0.07	0.80±0.05	1.5*0.9	1.5*1.1
	1000mg/kgbw	5.30±1.17	1.50±0.09	0.73±0.03	1.5*0.6	1.6*0.7
	2000mg/kgbw	4.67±0.55	1.66±0.13	0.84±0.05	1.5*0.9	1.6*1.1

Table XI: depicts that all organ weights are normal range in both the sexes in drug-treated groups compared to control group weights.

4. DISCUSSION

Ficus racemosa (cluster fig) has been extensively used in the traditional system of medicine for treating different ailments[18]. *Kalanchoe pinnata* (Bryophyllum pinnata) is also used by many tribal and herbal practitioners to treat different disorders, making it known as a wonder plant or divine plant[19]. In the present scenario, natural compounds and exploring their role in treating or preventing diseases are dominating the research interests. Even though several studies are present on these compounds, most are observational, descriptive, or phenomenal. More mechanistic studies are required on their constituent's pharmacokinetics, and their effects on consumption over a long duration of time are required[20-25]. In addition, several studies are present on different activities of various parts of FR and KP, but systemic evaluation of their leaves need to be done. In this assessment of systemic study, safety evaluation, and different activities, phytochemical screening and acute and sub-acute toxicity studies are taken up, which serves as the initial step to begin. The phytochemical evaluation of the hydroalcoholic leaf extract of FR and KP demonstrates that they contain alkaloids, carbohydrates, tannins, steroids, glycosides, saponins, and phenols (Table –II). It is reported that the ethanolic extract of bark and leaves of FR shows analgesic activity[26]. The ethanolic extract of leaves has been evaluated in CCl4-induced hepatotoxicity and was found to be hepatoprotective evident by the levels of SGOT, SGPT, and serum bilirubin[27]. The ethanolic extract also exhibited antioxidant activity evaluated by DPPH, superoxide radical scavenging assays[28]. Bryophyllol, bryophyllone, bryophollenone, bryophynol, bufadienolide- bryophyllin A, and Bryophyllin C are found to be the most important constituents of leaves of KP[29]. The leaves are also found to contain amino acids like thiamine, pyridoxine, ascorbic acid, glycine, cysteine, and nicotinamide[30]. The results of the acute toxicity study of hydroalcoholic extracts of FR and KP clearly indicate that both are acutely safe, and their LD50 is >2000mg/Kg body weight. A similar study evaluating the hydroethanolic extract of Kalanchoe brasiliensis (Crassulaceae) Leaves revealed a similar trend[31]. The sub-acute studies, which were conducted for 28 days, involved repeated administration of extracts of different doses and suggested that the extracts are safe when taken for a longer duration of time in both male and female rats. No abnormality was seen in body weight, feed consumption as well as the behavior of animals. Test animals' hematological and biochemical parameters were all comparable to their control groups. It is similar to the trend obtained in a study by Uche Mercylyn Ezinneand Chinyere Godwin Chinyere on phytochemical screening and sub-acute toxicity studies of aqueous leaf extract of ficus aurea from south-east nigeria[32]. The necroscopic studies also suggest no abnormality in organs determined by their appearance, histopathological examination, and organ weight. The results from the acute and sub-acute studies suggest that the extracts are safe with no harmful effects in rats.

5. CONCLUSION

Due to the Presence of various phytochemicals, FR and KP show various pharmacological activities. The whole plants and stems have been experimentally used, but the leaves still need to be explored. Nevertheless, it has sparked research interest. The present study concludes that both the leaves of *Kalanchoe pinnata* and fruits of *Ficus racemosa* have abundant phytochemicals and are safe both in acute and sub-acute studies. However, further research is required to explore and evaluate different pharmacological activities.

6. AUTHORS CONTRIBUTION STATEMENT

A.Naga Teja Pavani has collected the data and literature, conducted the experiments, analyzed the data and results, and prepared the original draft of the paper. Dr.D.Sheela has conceptualized and designed the study. She also reviewed the original draft prepared by A.Naga Teja Pavani and gave valuable input. Dr. L. Ramesh has verified the data literature and results thoroughly. He has also gone through the prepared manuscript. Dr.G.D.Sireesha helped trace the related works of the past and gave valuable inputs in preparing the manuscript. All authors have read and approved the final version of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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