Original Article

# In-Vitro Anti-Lipase And Antioxidant Activities Of Kalanchoe Pinnata Leaves And Ficus Racemosa Fruit Extracts

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## Abstract

**Introduction:** Herbal medicine has a long history of effective use across the world for wide range of medical conditions, and is increasingly seen as a potential area of research and development. This "Divine Plant" Kalanchoe pinnata and Ficus racemosa has a high therapeutic index because of their chemical composition and pharmacological activities.
**Methods:** We gathered Kalanchoe pinnata leaves and Ficus racemosa fruits from local market and verified by Dr.K.Madhava chetty Assistant professor, Department of Botany S.V University Tirupati. Antioxidant activity of both plant extracts were evaluated using invitro DPPH radical scavenging activity. In addition pancreatic lipase inhibition activity of both plant extracts were also assessed.
**Results:** Results obtained for FR extract and KP extracts showed that both exhibit considerable DPPH radical scavenging ability on a dose-dependent basis, proving their usefulness as test chemicals. It was found that the DPPH RSA IC50 value was significantly lower for the FR extract than the KP extract, indicating that the FR extract was more effective at scavenging DPPH radicals. In addition, Kalanchoe Pinnata leaves and Ficus racemosa fruits also showed significant pancreatic lipase inhibition. Pancreatic lipase inhibition is more significant with Kalanchoe pinnata when compared to Ficus racemosa.
**Conclusion:** the present study concludes that hydroalcoholic extracts of Kalanchoe pinnata leaves and Ficus racemosa fruits exhibit significant antioxidant activity as well as pancreatic lipase inhibitory activity. Further research is required to explore and evaluate different pharmacological activities.

**Keywords:** Antilipase, antioxidant, kalanchoe pinnata, Ficus racemose

## Introduction:

Medicinal herbs have been used successfully for thousands of years to treat a wide variety of illnesses, and they are now widely recognised as a promising new frontier in healthcare. The chemical makeup of the leaf, stem, and root of this "Wonderplant" or "Divine Plant" gives it a high therapeutic index [1. 2].
There is hope that the wealth of data offered here on the plant's phytochemical ingredients and the varied biological features of extracts and the constituents will encourage further study into its potential medical and agricultural applications [3]. Raw ingredients for phytochemicals are derived from B. Pinnatum by a few small enterprises in India and the Amazon. [4]. To confirm conventional wisdom in the context of a rational Phytotherapy on the toxicity of plant, and particularly on bufadienolides and their usage during pregnancy, more scientific evaluation of the separated principles and B. Pinnatum is required using specialised animal models. This overview of K. pinnata's pharmacological uses is a useful tool for uncovering more about this valuable plant. From the beginning

of time, people everywhere have turned to nature for healing in the form of medicinal plants [5, 6].
Natural products having therapeutic characteristics have been linked to the widespread usage of herbal treatments and healthcare preparations, such as those documented in ancient scriptures like the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants [7]. Traditional medicine and the usage of medicinal herbs have been widely documented as constituting the normative basis for the preservation of health in the majority of underdeveloped countries. In addition, various medicines and chemotherapeutics have been extracted and developed from these plants and from traditionally used rural herbal remedies, leading to a growing reliance on the usage of medicinal plants in industrialised nations. WHO reports that around 80% of the global population relies on botanical medicine for their primary health care [8].
The evergreen, lactiferous, deciduous Ficus racemosa Linn. (Moraceae) tree can grow up to 15–18 metres in height and has no noticeable aerial roots. Over 700 species belong to the genus Ficus, which is a pan-tropical genus with a wide distribution in the warmer regions of Asia, Africa, the Americas, and Australia [9]. Its distinctive reproductive system, which includes synconia fig and specialist pollinator wasps, allows it to be clearly distinguished from other plant groups and justify its continued classification as a single, big genus. All parts of the F. racemose plant are considered medicinally essential in Ayurveda, and the root, leaves, stems, and flowers have all been used to treat biliary diseases, jaundice, dysentery, diabetes, diarrhoea, and inflammatory illnesses [10].

## Material and Method:

## Materials:

## 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 airtight container for further use. Initially, 100g of each dried plant material powder was macerated with hydro alcohol 60% ethanol for 7days

Porcine pancreatic lipase (Cat No: L3126, Sigma),Triolein 2. (Cat No:Y0001113, Sigma) L-alpha Lectin 3. (Cat No: 429415, Sigma), sodium sodium salt hydrate of taurocholic acid (Cat No: T4009, Sigma) Tris buffer 5. (Cat No: 648314, Sigma), Chloroform 6. (Cat No: C2432, Sigma), Reagent for copper (Cat No: 311405, Sigma) Bácocóproine (Cat No: 140910, Sigma), Anisole 9. (Cat No: 296295, Sigma) Meyer's Reeshape 120mg Orlistat Capsules, USP Meyer's Organics Pvt Ltd, Mumbai, India, Diphenyl-1-picrylhydrazyl DPPH-2,2- (Cat No:D9132, Sigma), Sodium ascorbate (Cat No:A92-902, Sigma), A pipettor and movable multichannel pipettes (Benchtop, USA), Methanol 14. (Cat No: 34860-1L-R, Sigma), DMSO (Sigma, #PHR1309), 50 ml centrifuge tubes (TORSON # 546043),Borosil Glass tubes of 10 ml (TORSON), serological pipettes of 10 ml (TORSON),Tips from 10 to 1000 ul (TORSON)

## Equipments:

1. 2-10, 10-100, and 100-1000 ml pipettes.
2. Plate reader, ELX 800 (BIOTEK, USA)

## Assay Controls:

1. An empty control (Only Methanol)
2. Reverse control (Only DPPH)
3. Positive control (10ug/ml of ascorbic acid plus DPPH).

**Table 1: Study samples**

| Sr. No. | Sample Name/Code |
|---|---|
| 1 | FR extract |

| | |
|---|---|
| 2 | KP extract |

## METHODS

## DPPH ASSAY

We gathered kalanchoe pinnate leaves and Ficus racemosa fruit from local market and verified by Dr.K.Madhava chetty Assistant professor, Department of Botany S.V University Tirupati.
The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) test is widely used for investigating antioxidant properties of natural products. This technique is attractive because, among other things, it is easy to use and highly sensitive. The hypothesis that a hydrogen donor is also an antioxidant underpins this test. Compounds that act as radical scavengers are the focus of this analysis. Here, the process by which DPPH takes hydrogen from an antioxidant is depicted in Figure 1. DPPH is one of the few organic nitrogen radicals that is both stable and widely available for purchase. The degree to which DPPH is reduced in test samples is indicative of an antioxidant effect. Keeping tabs on DPPH levels with a UV spectrometer has quickly become the standard because of its ease of use and reliability. Intense absorption by DPPH is seen at 517 nm (purple). Once hydrogen from an antioxidant is taken in, the colour changes from purple to yellow, and DPPH is formed. In terms of the quantity of hydrogen atoms consumed, this reaction is stoichiometric. Therefore, the reduction in UV absorption at 517 nm is a simple indicator of the antioxidant action.

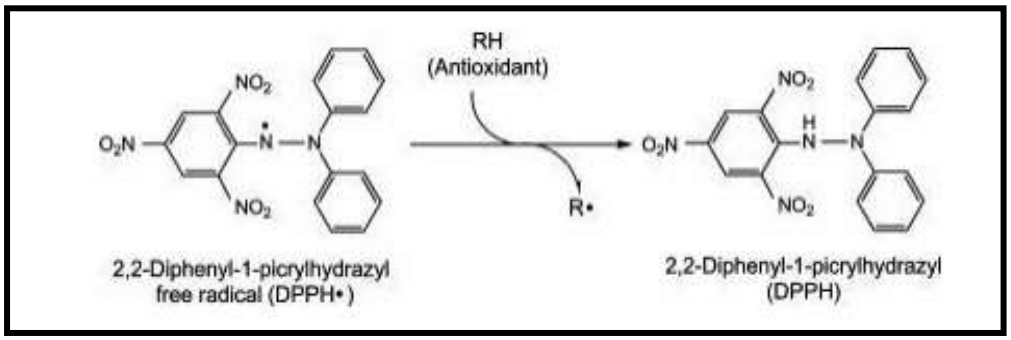


**Figure 1. Antioxidant compound conversion to DPPH free radical**

## Concentrations:

These findings are from an experiment where two chemicals were used to see if they could inhibit the alpha Glucosidase enzyme. This study used the following chemical concentrations:
The scavenging efficiency of test compounds against DPPH radicals was investigated. Concentrations of the chemicals employed in the study were as follows:

**Table 2: Test substances with varying doses and controls utilised in the study**

| Sr. No | Test Compounds | Concentration used |
|---|---|---|
| 1 | DPPH alone | 0.1Mm |
| 2 | Standard (Ascorbic acid) | 10ug/ml |
| 3 | Blank | Methanol |
| 4 | FR extract | 0.1, 1, 10, 100 and 1000ug/ml |
| 5 | KP extract | 0.1, 1, 10, 100 and 1000ug/ml |

**Procedure:**

The effect of extracts on DPPH radical was determined using the method of Md. Mahbubur Rahman, Md. Badrul Islam et.a [11]. Freshly made DPPH solution in methanol was collected in test tubes and provided extracts before repeated dilutions (100 g/ml) were added until the final amount of each test tube was 1 ml.Cover the test tubes with aluminium foil and incubate them at room temperature for 30 minutes without any light. Methanol was utilised as a blank and ascorbic acid served as the standard. A control sample with the same volume but no extract was created (DPPH alone).Use a plate absorbance reader to measure the absorbance at 517 nm. The following formula was used to determine the supplied compound's percent radical scavenging activity:
%DPPH Radical Scavenging Activity= [(Abs of Control-Abs of Sample)/Abs of Control] × 100

## Pancreatic Lipase Inhibition Assay

## Procedure:

The effect of extracts on pancreatic lipase inhibition was evaluated using the method of Jian Zhang, Min-Jung Kang et.al [12]. The rate of oleic acid release from Tirolean was used to measure lipase activity. To dissolve the substrate, a suspension of 90 mol of Tirolean, 12.6 mol of lecithin, and 9.45 mol of taurocholic acid was sonicated for 10 min. To obtain different amounts of the test chemicals, Ficus racemosa extract and Pinnata leaf extracts were diluted in 0.1M tris buffer (pH-7.0) (mentioned in MS excel sheet).After mixing 100 l of substrate solution, 100 l of sample solution, and 50 l of pancreatic lipase (2 mg/ml in Tries buffer), the reaction was incubated at 37 °C for 30 minutes. The mixture was shaken for 5 minutes while being extracted with 1ml of chloroform n-heptane (1:1) containing 2% methanol. After centrifuging the mixture at 1000 g for 5 minutes, the top aqueous phase was suction-extracted. The lower organic phase was then given a 700 l addition of copper reagent. The mixture was then centrifuged at 1000 g for 5 minutes after being shaken for 5 minutes, and 0.5 ml of the upper organic phase—which contained the copper salts of the extracted fatty acid—was then treated with 0.5 ml of a solution of bathocuproine in chloroform containing 0.1% bathocuproine and 0.05% 3-(2)-tertbutyl-4-hydroxyanisol.Distribute 200ul of each component to each well in a 96-well plate, and then use an ELISA reader to measure the absorbance at 490 nm.

The following formula was used to compute the percentage of lipase inhibition (I %):
I%=100-(As-Ab)/ (Ac-Ab) X 100,
Whereas is the average absorbance of the Sample
Ab is the average absorbance of the Blank
Ac is the average absorbance of the Control.

**Table 3: Study Samples**

| Sl. No. | Sample Name/Code | Concentrations |
|---|---|---|
| 1 | Ficus racemosa extract | 5 (0.1, 1, 10, 100, 1000ug/ml) |
| 2 | Kalanchoe Pinnata leaves | 5 (0.1, 1, 10, 100, 1000ug/ml) |
| 3 | Orlistat | 5 (0.1, 1, 10, 100, 1000uM/ml) |

## Concentrations:

Two test compounds (TCs) are employed to determine the degree to which Lipase enzyme activity is reduced. Concentrations of the chemicals employed in the study were as follows:

**Table 4: Study Std. and Sample concentrations**

| Sl.No | Test Compounds | Concentration treated to cells |
|---|---|---|

| | | |
|---|---|---|
| 1 | Untreated | No treatment |
| 2 | Standard (Orlistat) | 5 (0.1, 1, 10, 100, 1000uM/ml) |
| 3 | Blank | Only Tris buffer |
| 4 | Ficus racemosa extract | 5 (0.1, 1, 10, 100, 1000ug/ml) |
| 5. | Kalanchoe pinnata | 5 (0.1, 1, 10, 100, 1000ug/ml) |

## Results:

**Table 5: FR and KP extract dose-dependent DPPH % inhibition values and IC50 concentrations**

| **Overlaid %DPPH RSA Inhibition with IC50 values** | | |
|---|---|---|
| Concentration (µg/ml) | FR extract | PL extract |
| DPPH alone | 0.00 | 0.00 |
| Ascorbic acid-10ug/ml | 34.89 | 34.89 |
| 0.1ug/ml | 0.16 | 0.16 |
| 1ug/ml | 6.00 | 3.12 |
| 10ug/ml | 28.12 | 17.76 |
| 100ug/ml | 74.22 | 43.54 |
| 1000ug/ml | 97.20 | 83.41 |
| IC50 (ug/ml) | **31.87** | **334.95** |

## DPPH radical scavenging study of the extracts:

Data from an ELISA reader and a spectrophotometer indicate that both the FR extract and the KP extract inhibited DPPH RSA activity, with respective IC50 values of 31.87 and 334.95 g/ml. Ascorbic acid (at a concentration of 10ug/ml) was utilised as the standard control in the experiment.

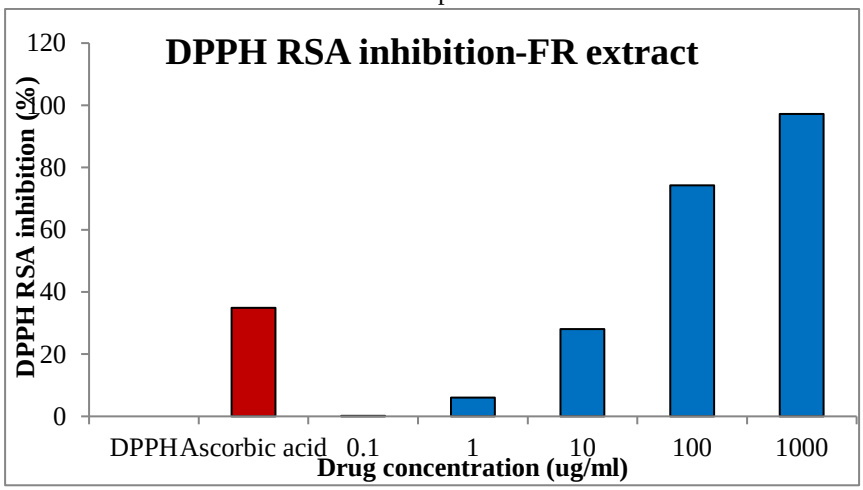


**Figure 2: Ficus racemosa extract DPPH-RSA activity**

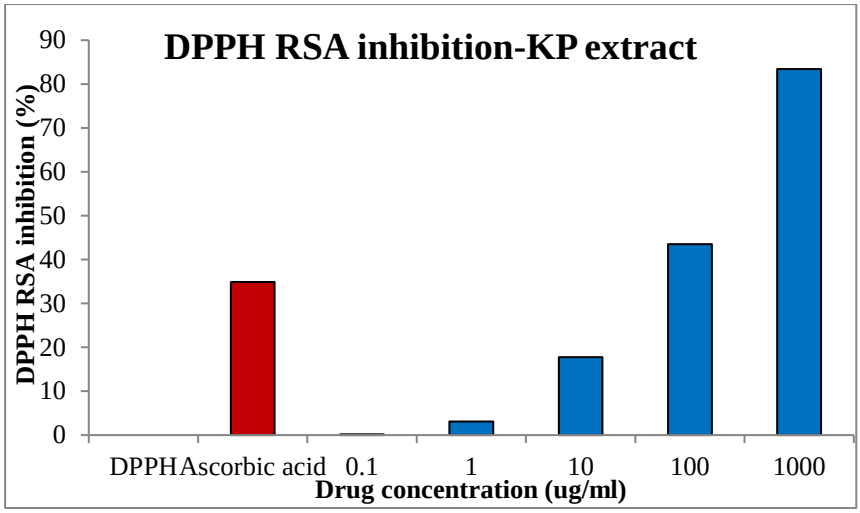


**Figure 3: KP extract DPPH RSA**

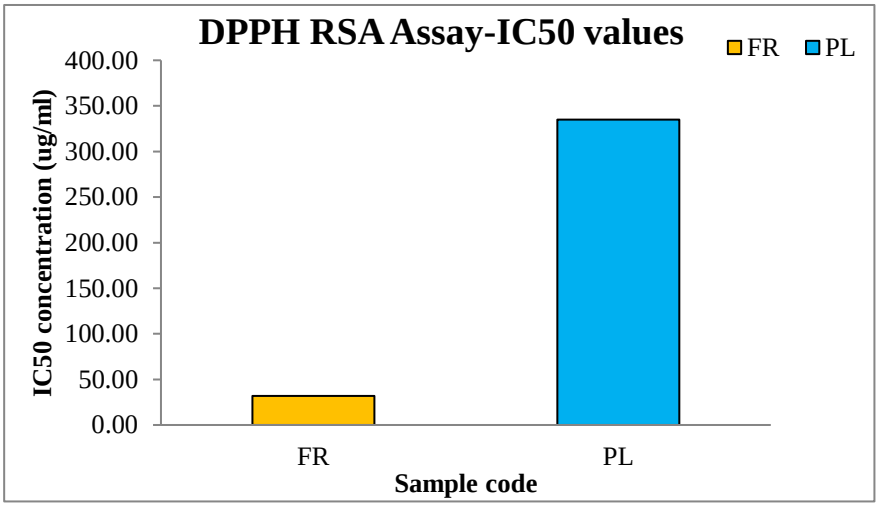


**Figure 4: FR and KP extract DPPH IC50 values**

## Pancreatic lipase enzyme inhibition study:

Based on the results of an ELISA Reader investigation on the inhibition of the lipase enzyme, it appears that the test compounds exhibited effective dose-dependent inhibition of lipase. MS Excel data files including the observed absorbance values were included as a companion to the report.

**Table6: In vitro lipase inhibitory activity of Orlistat at various doses. Concentration-dependent inhibition increased significantly**

| Drug conc (uM/ml) | % Lipase inhibition | IC50 conc (uM/ml) |
|---|---|---|
| 0.1 | 5.56 | **4.09** |
| 1 | 12.67 | |
| 10 | 72.47 | |
| 100 | 83.52 | |
| 1000 | 91.85 | |

**Table 7: Different doses of Ficus racemosa extract inhibit lipase in vitro. Concentration-dependent inhibition increased significantly**

| Drug conc (ug/ml) | % Lipase inhibition | IC50 conc (ug/ml) |
|---|---|---|
| 0.1 | 1.18 | **137** |
| 1 | 5.20 | |
| 10 | 9.05 | |
| 100 | 48.46 | |
| 1000 | 87.65 | |

**Table 8: Different doses of Kalanchoe Pinnata leaf extract inhibit lipase in vitro. Concentration-dependent inhibition increased significantly**

| Drug conc (ug/ml) | % Lipase inhibition | IC50 conc (ug/ml) |
|---|---|---|
| 0.1 | 5.53 | **39.32** |
| 1 | 12.60 | |
| 10 | 30.01 | |
| 100 | 64.82 | |
| 1000 | 91.56 | |

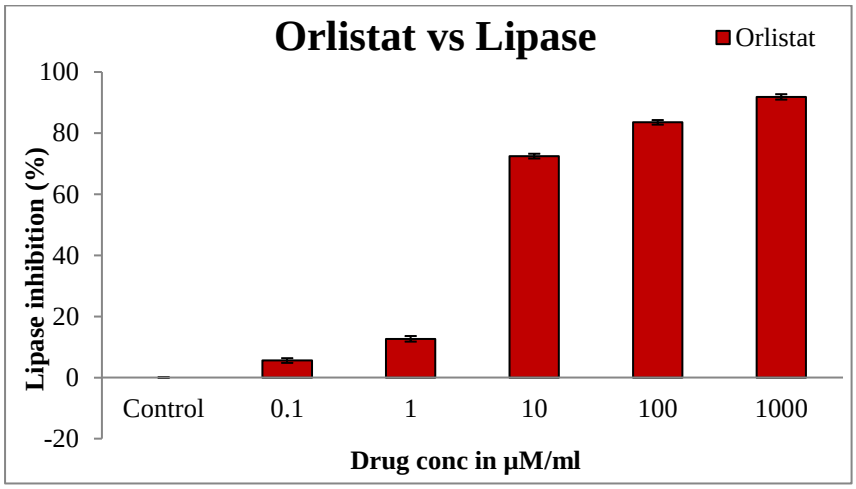


**Figure 5: Orlistat inhibited lipase at various amounts**

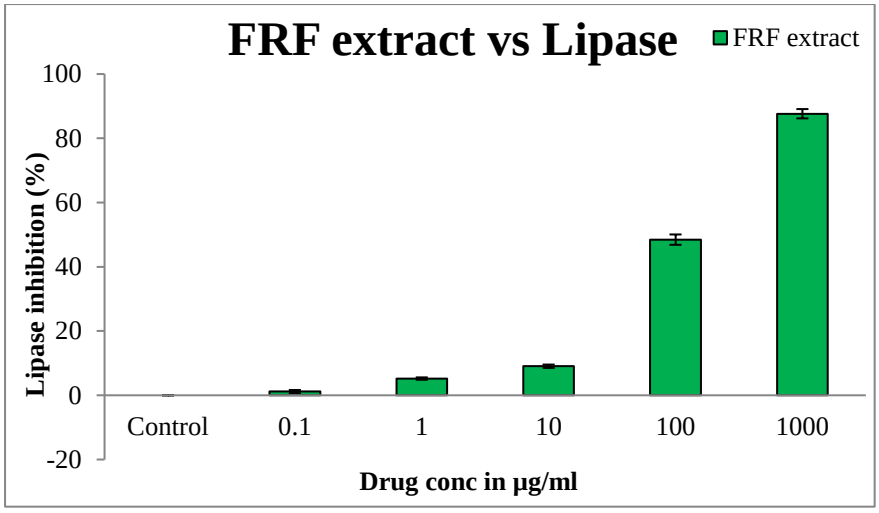


**Figure 6: FRF extract inhibited lipase at various doses**

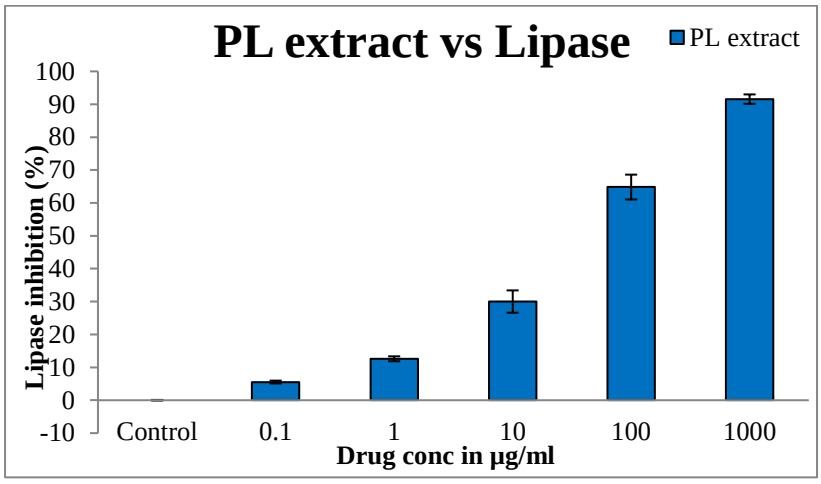


**Figure 7: Different doses of KP extract inhibited lipase**

## Discussion:

Kalanchoe pinnata also known as miracle plant has been used by many herbal and tribal practitioners for treating various disorders making it to be known as divine plant [13].The plant Ficus racemosa as a whole has various therapeutic indications and is having an important sacred role in homas and yagnas [14]. In recent study conducted by F. Janeeta Priya et.al on Kalanchoe pinnata extract proved that synthesis of silver nanoparticles using plant extract is energy efficient and bio-hazardous chemical synthesis [15]. Even though several studies are present on these compounds [19-21], there is need to explore various activities of these plants by doing extensive research. In light of this the present study is taken up to evaluate antioxidant and antilipase activity of both plant extracts. The results that were obtained in the present study demonstrated that both hydroalcoholic extracts of FR and PL have a significant dose-dependent ability to scavenge DPPH radicals, which showed their efficacy as test chemicals. It was found that DPPH RSA IC50 values for FR and KP were 31.87 and 334.95 ug/ml respectively (Table 5) which indicates FR is having significant DPPH radical scavenging activity when compared to KP. In addition to DPPH radical scavenging activity the present study also demonstrated pancreatic lipase inhibition of both extracts. KP extract exhibited significant antilipase activity when compared to FR extract. IC50 values for FR and KP were 137 and 39.2 ug/ml respectively. (Table 7, 8)

Previous study conducted by Miraj S, et al., on antimicrobial, antioxidant and cytotoxic activities of three different plant extracts showed similar trends of the present study and also they established via extensive pharmacological research that phytochemicals including flavonoids, alkaloids, and saponins are molecules

responsible for anti-inflammatory and anti-oxidant properties and they offer potential as a therapy for cardiovascular disease [22]. They also counteract the effects of reactive oxygen species and free radicals.

The results obtained in the present study are similar to other study conducted by Sohaib M. et al., and Satija S. et al., which revealed phytochemical extracts of ferns have strong anti-oxidant activity. Two other ferns were also included in their study, but only A. veneris fared better [23]. Report on synergistic effect of conventional medicinal herbs against different pharmacological activity supports the analysis of present study [24]

Rini Jarial et.al Using in vitro assay techniques, the haemolytic activity, PPL inhibition, cholesterol degradation, and anti-oxidant activity of fern MFE were analysed. Their research also demonstrated that A. veneris MFE inhibits mammalian lipase function in vitro.

Anti-lipolytic activity against PPL was 76.5%, while cholesterol oxidase inhibition was 79.0% in the MLE. As a result, their study provided strong experimental evidences that the MFE of A. veneris is a promising source of compounds with potential natural anti-oxidant and anti-bacterial activities, as well as efficient cholesterol degradation and antilipolytic potential that might be beneficial in weight management and obesity-related diseases, In light of this, efforts have been made to investigate and identify potent pancreatic lipase inhibitory drugs in the hopes of developing a more secure anti-obesity therapy. Numerous fern-derived bioactive phytochemicals have been tested for their ability to combat obesity, which could lead to the exciting discovery of novel phyto-constituents with manageable toxicity and few unintended consequences [25], which strongly supports the results of our present study.

## Conclusion:

Results obtained for FR extract and PL extract showed that both exhibit considerable DPPH radical scavenging ability on a dose-dependent basis, proving their usefulness as test chemicals. It was found that the DPPH RSA IC50 value was significantly lower for the FR extract than the PL extract, indicating that the FR extract was more effective at scavenging DPPH radicals. The IC50 values for Ficus racemosa extract and Pinnata leaf extracts, respectively, were 31.87 and 334.95 ug/ml respectively. In addition,Ficus racemosa fruit and Kalanchoe pinnata leaf extracts has been shown to inhibit Lipase with an IC50 values of 137 and 39.2 ug/ml respectively which is comparable with standard Orlistat.

## Conflict of Interest:

None


## Funding Support:

Nil


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