

In vitro enzymatic investigation of *Ficus carica* (Fruit)

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Abstract: The present study demonstrates the biological study of *Ficus carica* fruit. Methanolic extract of plant fruit was prepared and evaporated under reduced pressure by rota vapor and n-hexane, Chloroform, ethyl acetate and n-butanol soluble fractions were prepared separately from crude methanolic extract. These fractions were then screened for acetyl cholinesterase, butyryl cholinesterase and lipoxygenase activities. n-butanol soluble fraction showed significant antiacetylcholinesterase activity ($78.55 \pm 0.76\%$) with IC_{50} of $55.8 \pm 0.37 \mu\text{g/ml}$, ethyl acetate soluble fraction showed significant anti-butrylcholinesterase activity ($70.35 \pm 0.85\%$) with IC_{50} of $276.5 \pm 0.64 \mu\text{g/ml}$ and significant anti-lipoxygenase activity was shown by ethyl acetate soluble fraction ($62.52 \pm 0.26\%$) with IC_{50} of $380 \pm 0.08 \mu\text{g/ml}$.

Keywords: *Ficus carica*, anti-acetyl cholinesterase, butryl cholinesterase, lipoxygenase.

INTRODUCTION

For centuries, plants have been used as a major source of medicines. Traditionally, crude drugs have been employed as medicines (Balick and Cox, 1997). *Ficus carica* belongs to the family Moraceae. It is commonly called as Fig and grows in tropical and sub-tropical regions. It has traditionally been used for the cure of different diseases. *Ficus* constitute the largest genera of the medicinal plants with about 750 species (Berg, 2001). Figs are perhaps the oldest of all cultivated fruit species (Ensminger *et al.*, 1994). Medicinally important plants had been used in folk medicines for centuries (Kislev *et al.*, 2006). Fig is used in weakness, inflammation, thirst, paralysis, nose bleeding, piles, hair growth stimulation, and also acts as aphrodisiac, anti-pyretic, tonic and lithonotriptic (Kirtikar and Basu, 2005). It also possesses anti-diabetic, diuretic, anti-cancer, antioxidant, immune stimulation, anti-platelet, antipyretic, anti-inflammatory, anti-angiogenic, immunomodulatory and anti-microbial activities. *Ficus carica* has also been reported to contain many bioactive compounds (Oliveira *et al.*, 2009; Gibernau *et al.*, 1997).

MATERIAL AND METHODS

Collection and identification of plant material

Two kg of dried *F. carica* fruit was purchased from the Bahawalpur local market and was identified by Dr. Muhammad Shafique, Associate Professor, Department of botany, The Islamia University of Bahawalpur, Pakistan.

Extraction

Dried fruit was grounded and extracted successively in methanol for 15 days. Filtrate was concentrated under

reduced pressure by Rotavapor-R20 at 38°C and finally crude extract obtained was further fractionated in to n-hexane, chloroform, ethyl acetate and n-butanol soluble fractions. All chemicals used in the above procedure were purchased from Merck Group Frankfurter Strabe 25064293, Darmstadt, Germany.

Enzymes inhibition assays

Acetyl cholinesterase and Butrylcholinesterase inhibition assay

Acetyl cholinesterase and Butrylcholinesterase inhibition assay was performed according to (Ellman *et al.*, 1961) with slight modifications. Test compound ($10 \mu\text{l}$) 0.5 mM well⁻¹, $60 \mu\text{l}$ Na_2HPO_4 buffer 50 mM and pH 7.7 and $10 \mu\text{l}$ ($0.5 \text{ unit well}^{-1}$) butrylcholinesterase and acetyl cholinesterase enzyme was added in a 96 well-plate, separately. The Assay ingredients were blended and 405 nm was selected for pre-read. The material was incubated at 37°C for 10 minutes. $10 \mu\text{l}$ of 0.5 mM well^{-1} reactant (butryl thiocholine chloride) was combined trailed by adding of $10 \mu\text{l}$ DTNB 0.5 mM well^{-1} reaction was started. It was then placed in incubator after 30min at 37°C and value was noted at 405 nm by using 96-well plate reader, Biotek, Synergy HT, USA. Eserine was used as standard. All analyses were evaluated in triplicate. Following equation was used to measure the enzyme % inhibition

$$\% \text{ inhibition} = (\text{abs of sample} / \text{abs of control}) * 100$$

Lipoxygenase inhibition assay

Lipoxygenase inhibition assay was performed according to (Tappel *et al.*, 1953) with slight modification. $20 \mu\text{l}$ of the investigational compound was blended with $140 \mu\text{l}$ KH_2PO_4 buffer (pH 8.0, 100 mM) and $15 \mu\text{l}$ purified LOX enzyme (127 units) per well in a 96 well-plate. The contents of the assay were blended and incubated for 10min at 37°C . Pre-read was noted at 234 nm . The

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reaction was started by adding 25µl substrate solution. The variations in absorbance after 6-10 min at 234nm were measured. Baicalein was employed as standard. Measurements were recorded in triplicates and determined as mean \pm SEM. Assay determine the IC₅₀ readings in the test solution at various dilutions that is 0.5, 0.125, 0.25, 0.0313, 0.015, 0.0625 mg. The outcomes were worked out on Amherst USA software, Ez-fit5 Perrella Scientific Inc.

Following equation was used to measure the enzyme % inhibition %age inhibition = (abs. of sample/abs. of control)*100

STATISTICAL ANALYSIS

Statistics were presented as mean \pm S.D of three replicates and evaluated by SPSS version 15. Tukey Post-Hoc one way analysis of variance (ANOVA) was utilized for the determination of alterations among mean. Values of P<0.05 were considered as significant.

RESULTS

Enzymatic screening

Enzyme inhibition assays of different soluble fractions and methanolic extract of *Ficus carica* fruit was subjected to screening.

Enzymes inhibition assays

Butryl-cholinesterase, Acetyl cholinesterase and Lipoxigenase enzyme inhibitions activities were carried on *Ficus carica* fruit.

In vitro acetyl cholinesterase and Butrylcholinesterase inhibition assay

Results demonstrated that significant acetyl cholinesterase inhibition was revealed by n-butanol soluble fraction (78.55 \pm 0.76%) with IC₅₀ of 55.8 \pm 0.37µg/ml showed by crude methanolic extract (70.83 \pm 0.97%) with IC₅₀ of 126.1 \pm 0.69µg/ml Negative values of chloroform soluble fraction, n-hexane soluble fraction and ethyl acetate soluble fraction, revealed that these may potentiate the enzyme AChE. Ethyl acetate soluble fraction showed significant BChE inhibition (70.35 \pm 0.85%) with IC₅₀ of 276.5 \pm 0.64 µg/ml and chloroform soluble fraction showed (69.97 \pm 0.32%) with IC₅₀ of 703.2 \pm 0.08µg/ml followed by crude methanolic extract (64.61 \pm 0.14%) with IC₅₀ of 270.1 \pm 0.78µg/ml and n-hexane soluble fraction (60.19 \pm 0.58%) with IC₅₀ of 205.4 \pm 0.35µg/ml and n-butanol soluble fraction (59.38 \pm 0.41%) with IC₅₀ of 291.1 \pm 0.15µg/ml.

In vitro Lipoxigenase inhibition assay

The outcomes of *Ficus carica* fruit revealed the significant Lipoxigenase inhibition of ethyl acetate soluble fraction (62.52 \pm 0.26) with IC₅₀ of 380 \pm 0.08µg/ml

Showed by crude methanolic extract (55.52 \pm 0.34) and n-hexane soluble fraction (47.14 \pm 1.56).

DISCUSSION

The butrylcholinesterase (BChE) and acetyl cholinesterase (AChE) are important biological enzyme (Vita *et al.*, 1990). Neurodegenerative Alzheimer's disease (AD) is caused by a decreased cholinergic activity (Sayer *et al.*, 2004). One of the approach for the management of Alzheimer's disease is the consumption of butryl-cholinesterase and acetyl cholinesterase for (Wilcock *et al.*, 2000). *Ficus carica* results of BChE and AChE inhibition activities are presented in table 1.

Polyphenolic contents are responsible for AChE and BChE inhibition (Ahmad *et al.*, 2013). Most abundant polyphenol was the Cyanidin-3-rutinoside (108.9mg/100g) and the second was quercetin-rutinoside (16mg/100g) (Vallejo *et al.*, 2012). Ahmed and Urooj in 2010 showed AChE inhibition of *Ficusracemosa*. It was determined that *Ficusracemosa* found in polyphenolic substances and these substances might be responsible for AChE inhibition (Ahmed & Urooj, 2010). Many adverse effects had been illustrated with anti-acetyl cholinesterase drugs (Turon-Estrada *et al.*, 2003). Therefore, the least toxic acetyl cholinesterase inhibitors drugs are considered as the need of the time. Hence, AChE inhibitory activities of plant extracts could be a possible source of emerging accepted medications for Alzheimer's disease (Atta-ur-Rahman *et al.*, 2000). The present work on *Ficus carica* extract has revealed significant AChE and BChE inhibitory effects. *Ficus carica* fruit exhibited the potential for the treatment of Alzheimer's disease. Additionally, the results pointed out the detection of lead compounds for Alzheimer's disease management. Isolation of the bioactive compounds responsible for AChE and BChE inhibition is another prime outcome of this study.

Schneider and Bucar described in 2005 pointed out that 5-Lipoxygenase was prone to have antioxidant property due to presence of non-heme iron atom at the active portion of the enzyme which experiences redox reaction for enzyme triggering. Synthesis of inflammatory mediators Inhibition of the (PGE2, PGF2 and PGD2) by restricting 5-Lipoxygenase and Cyclooxygenase-1 was well-thought-out as a strong management of diseases related to inflammation (Warner & Mitchell, 2004). Plants could assist as a basis of finding lead compounds for the production of further effective, less toxic and upright pharmacokinetic profile (Bohlin & Bruhn, 1999). The results of 5-Lipoxygenase inhibition activity of crude methanolic extract, n-hexane soluble fraction, ethyl acetate soluble fraction, chloroform soluble fraction and n-butanol soluble fraction have been presented in table 2.

Table 1: *Ficus carica* results of AChE and BChE inhibition activities

Fraction used	Conc.	AChE % inh	IC ₅₀ µg/ml	BChE % inh	IC ₅₀ µg/ml
Crude methanolic extract	1.0mg/ml	70.83±0.97 ^a	126.1±0.69	64.61±0.14 ^a	270.1±0.78
n-hexane soluble fraction	1.0mg/ml	-50.00±0.12 ^b	-	60.19±0.58	205.4±0.35
Chloroform soluble fraction	1.0mg/ml	-47.99±0.46 ^c	-	69.97±0.32 ^c	703.2±0.08
Ethyl acetate soluble fraction	1.0mg/ml	-51.01±0.53 ^d	-	70.35±0.85 ^d	276.5±0.64
n-butanol soluble fraction	1.0mg/ml	78.55±0.76 ^e	55.8±0.37	59.38±0.41 ^e	291.1±0.15
Eserine	0.25mMol/ml	91.29±1.18 ^f	0.04±0.0001 µMol/l	91.29±1.18 ^f	0.04±0.0001µMol/l

Table 2: *Ficus carica* Lipoxxygenase inhibition activity

No.	Sample used	Conc. (µg/ml)	LOX Value % inhibition	IC ₅₀ (µg/ml)
1.	Crude methanolic extract	0.5µg/ml	55.52±0.34 ^a	380±0.08
2.	n-hexane soluble fraction	0.5µg/ml	47.14±1.56 ^b	-
3.	Chloroform soluble fraction	0.5µg/ml	20.47±0.27 ^c	-
4.	ethyl acetate soluble fraction	0.5µg/ml	62.52±0.26 ^d	-
5.	n-butanol soluble fraction	0.5µg/ml	30.28±1.36 ^e	-
6.	Baicalein	0.5mM/ml	93.79±1.16	22.4±1.40µMol

Values are mean (n=3) ± standard deviation. Superscript a,b,c,d,e and f showed that mean± standard deviation in the similar column with altered superscript are significantly different (p <0.05)

Initiation of inflammation and its maintenance was mediated by Leukotriene's (Ammon *et al.*, 1993). In order to evaluate its anti-inflammatory activity, lipoxxygenase inhibitory effect of *Ficus carica* fruit was evaluated. In general, antioxidants inhibit lipoxxygenase enzyme (Ammon *et al.*, 1993). Studies have revealed that the process of inflammation was due to presence of free radicals and phenolic compounds might be acted as scavenger of free radical in the process of inflammation (Sreejayan & Rao, 1996). Antioxidant activities of plant extracts measured by DPPH and NO scavenging methods were found to be correlated with lipoxxygenase inhibition activities with correlation coefficients of 0.80 and 0.72, respectively (Trouillas *et al.*, 2003). Most potent lipoxxygenase inhibitors were flavonoids such as isoquercetin, quercetin, flavones, luteolin, or rhamnetin (Hiermann *et al.*, 1998). Major flavonoid found in *Ficus carica* was Luteolin and Quercetin (Vaya & Mahmood, 2006). 5-lipoxxygenase inhibition activity of *Ficus carica* was due to these flavonoid compounds. Moreover, fatty acids can act as lipoxxygenase inhibitors (Paubert-Braquet *et al.*, 1997) and linoleic acid has been detected in dried *Ficus carica* fruit by gas chromatography (Jeong and Lachance, 2001). Therefore, linoleic acid might be responsible for LOX inhibition by *Ficus carica* fruit.

CONCLUSION

Ficus carica (fruit) has shown different enzymatic activities like anti-acetyl cholinesterase, anti-butrylcholinesterase and anti-lipoxxygenase activities. Crude methanolic extract, n-hexane and ethyl acetate soluble fractions showed significant lipoxxygenase

inhibition activity. Crude methanolic extract and n-butanol showed moderate to high acetyl cholinesterase inhibition activity whereas n-hexane, ethyl acetate and n-butanol soluble fractions showed butrylcholinesterase inhibition activity.

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