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EVALUATION OF LIPOXIGENASE INHIBITORY ACTIVITY OF FRUITS OF *CITRULLUS COLOCYNTHIS*

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Abstract

The main objective of present investigation was to evaluate lipoxigenase inhibitory activity of *Citrullus colocynthis* **(**Kaurtumba; Cucurbitaceae) fruits obtained from wild areas of Rajasthan, Punjab and Haryana using standardized *in vitro* spectrophotometric model. The various extracts (petroleum ether, acetone, ethanol and water extracts) of plant were prepared separately in the increasing order of polarity using Soxhletion. The ethyl acetate fractions were prepared separately from ethanol extract of respective plant using reflux technique. Preliminary phytochemical screening showed showing presence of bioactive classes of phytoconstituents (flavonoids and phenolic compounds) only in acetone extract, ethanol extracts and ethyl acetate fraction. Therefore, acetone extract, ethanol extract and ethyl acetate fraction were selected for evaluation of lipoxigenase inhibitory activity. The ethyl acetate fraction of each plant sample exhibited strong lipoxygenase inhibitory activity followed by respective ethanol extract and acetone extract, as compared to standard lipoxygenase inhibitory drug quercetin. The ethyl acetate fraction of Rajasthan variety (IC_{50} = 59.26 µg/ml) exhibited strong lipoxygenase inhibition activity followed by Punjab variety (IC_{50} = 109.95 µg/ml) and Haryana variety (IC_{50} = 133.62 µg/ml), as compared to quercetin (IC_{50} = 26.28 µg/ml). The various scientific reports available online suggested that flavonoids and phenolic compounds such as caffeic acid, quercetin, rutin, kaempferol and apigenin have been used as potential lipoxigenase inhibitory agent. Therefore, finally it can be suggested that our findings of plant against lipoxigenase enzyme may be due to presence of due to presence of flavonoids and phenolic compounds.

Keywords: *Citrullus colocynthis*, Kaurtumba, Lipoxigenase inhibitory, Quercetin.

Introduction

The main objective of lipoxygenase enzyme was to convert polyunsaturated fatty acid such as arachidonic and linoleic into active species responsible for various diseases such as inflammation, allergy, diabetes, pain, cancer, oxidative stress and asthma. These disorders are responsible for increased production of leukotrienes. The increased levels of leukotrienes are observed in rheumatoid arthritis, allergic rhinitis and asthma. The higher levels of leukotrienes are decreased or prevented by inhibition of lipoxygenase enzyme (Catalano *et al*., 2005; Pidgeon *et al*., 2007). Traditionally, *Citrullus colocynthis* fruits have been used in the treatment of inflammation, diabetes and cancer may be via inhibition of lipoxygenase enzyme. Therefore, *Citrullus colocynthis* fruits are selected for the present investigations.

Citrullus colocynthis (L.) Schrad is commonly known as Kaurtumba, belonging to family Cucurbitaceae. The plant is generally accessible in the Sahara and Arabian deserts, Sudan and Southern piece of Asia including Pakistan, India and Southern Islands (Perveen *et al*., 2007). Traditionally, this plant have been used the treatment of various ailments such as diabetes, cancer, obstruction, sickness, asthma, bronchitis, jaundice, joint pain and mastitis (Chopra, 1958; Abo *et al*., 2008). The plant has been reported to contain various types of chemical constituents such as cucurbitacins (Chen *et al*., 2005); flavonoids and phenolic acids compounds – catechin, kaempferol, gallic acid, caffeic acid (Meena and Patni, 2008; Hussain *et al*., 2013); alkaloids - choline (Sayed *et al*., 1973); volatiles oil / terpenoids (Gurudeeban *et al*., 2011) and fatty acids – palmitic acid, stearic acid, linoleic acid, oleic acids (Sawaya *et al*., 1983); tocopherols

Table 1: The results of percentage yields of various extracts and fractions.

C. colocynthis	Type of extract/fraction	Percent yield (w/w)
Rajasthan	Petroleum ether	3.45
	Acetone	8.55
	Ethanol	10.25
	Water	7.25
	Ethyl acetate fraction	18.25
Punjab	Petroleum ether	3.30
	Acetone	8.25
	Ethanol	10.10
	Water	7.01
	Ethyl acetate fraction	18.10
Haryana	Petroleum ether	3.05
	Acetone	8.11
	Ethanol	9.88
	Water	6.85
	Ethyl acetate fraction	17.55

*Ethyl acetate fraction was prepared from ethanol extract.

and carotenes – α-tocopherol, γ-tocopherol, β-carotene (Kalhoro *et al*., 2002). The major pharmacological activities of plant have been scientifically reported such as anti-inflammatory, analgesic (Marzouk *et al*., 2010; Marzouk *et al*., 2011a; Marzouk *et al*., 2011b), antidiabetic (Huseini *et al*., 2009) and cancer (Tannin-Spitz *et al*., 2007).

But, this plant has not been investigated for lipoxigenase inhibitory activity till date. Thus, the present research work was planned to evaluate lipoxigenase inhibitory activity of *Citrullus colocynthis* fruits obtained from wild areas of Rajasthan, Punjab and Haryana using standardized *in vitro* spectrophotometric model.

Materials and methods

Authentication of plant materials

The exhaustive dried fruits of *Citrullus colocynthis* for present research work were collected from wild regions of different states such as Rajasthan, Punjab and Haryana. The botanical identity of collected dried fruits of *Citrullus colocynthis* was confirmed before starting the research work by Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar (Punjab) India with registration number of certificate 1164 dated 11/01/2019.

Chemicals, reagents, solvents and enzymes

The laboratory grade chemicals, reagents and solvents of E Merck, Delhi, India and S.D. Fine Chemicals, Mumbai, India were used in extraction and preliminary phytochemical studies. The analytical grade chemicals, reagents and solvents of E Merck, Delhi, India and S.D. Fine Chemicals, Mumbai, India were used in spectrophotometric studies. Lipoxigenase enzyme was procured from Sigma-Aldrich, USA.

Preparation of various extracts and fractions

The various extract such as petroleum ether, acetone, ethanol and water extracts of *Citrullus colocynthis* fruits collected from different parts of country were prepared separately using Soxhletion technique as per standardized procedure available online. Similarily, the ethyl acetate fraction of each plant sampe was prepared separtely from their respective ethanol extract using reflux technique as per standardized procedure available online (Richa *et al*., 2017). The various extracts and fractions were subjected to preliminary phytochemical testes for identification of phytochemical nature (Farnsworth, 1966).

Table 2: Preliminary phytochemical screening of various extracts and fractions.

Phytoconstituents	*C. colocynthis* (extract/fraction) Rajasthan/Punjab/Haryana				
	Petroleum ether	Acetone	Ethanol	Water	Ethyl acetate
Free Reducing Sugars	-/-/-	—/—/—	-/-/-	+/+/+	-/-/-
Proteins & Amino Acid	-/-/-	-/-/-	-/-/-	+/+/+	-/-/-
Terpenoids	-/-/-	+/+/+	-/-/-	-/-/-	-/-/-
Sterols	+/+/+	-/-/-	-/-/-	-/-/-	-/-/-
Tannins	-/-/-	+/+/+	++/++/++	-/-/-	++/++/++
Phenols	-/-/-	+/+/+	++/++/++	-/-/-	++/++/++
Alkaloids	-/-/-	+/+/+	-/-/-	-/-/-	-/-/-
Glycosides	-/-/-	-/-/-	+/+/+	-/-/-	+/+/+
Flavonoids	-/-/-	-/-/-	+/+/+	-/-/-	+/+/+
Saponins	-/-/-	-/-/-	+/+/+	-/-/-	+/+/+
Starch	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
Gums and mucilage	+/+/+	-/-/-	-/-/-	-/-/-	-/-/-
Fixed oils and fats	+/+/+	-/-/-	-/-/-	-/-/-	-/-/-
Cyanogenetic glycoside	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-

Table 3: The results of lipoxigenase inhibitory activity of various extracts and fractions. (n = 3)

Treatment	Concentration (µg/ml)	% lipoxigenase inhibitory activity(Mean ± S.D.) Rajasthan	% lipoxigenase inhibitory activity (Mean ± S.D.) Punjab	% lipoxigenase inhibitory activity(Mean ± S.D.) Haryana
Quercetin	2	17.52±2.15		
	4	20.12±3.89		
	8	25.36±3.58		
	16	38.44±3.35		
	32	60.12±1.32		
	64	98.45±0.90		
Acetone extract	31.25	31.48±2.01	28.45±1.68	26.45±2.25
	62.5	33.50±1.89	30.40±2.45	29.48±2.40
	125	37.85±2.11	33.59±1.10	31.24±1.48
	250	41.56±1.58	39.78±1.89	37.58±1.56
	500	51.25±2.25	47.57±3.24	46.58±3.54
	1000	75.58±3.48	70.48±3.47	71.25±2.47
Ethanol extract	31.25	44.58±3.01	42.45±2.25	40.12±3.10
	62.5	47.50±2.45	43.89±2.65	42.48±2.88
	125	51.35±2.60	47.59±3.78	44.59±2.45
	250	55.48±2.05	53.12±3.01	50.25±3.56
	500	65.24±2.68	62.54±3.45	57.58±1.58
	1000	85.47±3.45	80.44±3.02	75.89±1.95
Ethyl acetate fraction	31.25	47.89±3.20	45.45±2.88	44.50±3.15
	62.5	49.87±2.48	47.80±3.56	46.52±3.45
	125	52.45±3.01	51.59±2.80	51.20±2.01
	250	60.48±1.99	57.45±2.45	55.65±1.45
	500	68.69±2.48	65.48±2.65	64.58±2.20
	1000	90.47±2.89	88.36±2.48	85.47±3.99

Lipoxigenase inhibitory activity studies

The various extracts and fractions were subjected lipoxygenase inhibitory activity using well designed spectrophotometric method available online (Haq *et al*., 2004). The quercetin was used as standard lipoxygenase inhibitory drug. The raw data of activity is presented in the form of mean ± S.D. and IC_{50} value. The each experiment is performed in triplicate.

Results and Discussion

The various extracts and fractions of fruits of *Citrullus colocynthis* obtained from wild areas of different states Rajasthan, Punjab and Haryana were prepared using Soxhletion process. The results of percentage yield (w/w) are depicted in table 1.

The results of preliminary phytochemical screening for presence of different classes of phytoconstituents in fruits of *Citrullus colocynthis* obtained from wild areas of different states Rajasthan, Punjab and Haryana showing same classes of phytoconstituents in respective extracts. The results of preliminary phytochemical screening are depicted in table 2.

Table 4: The results of IC_{50} values of various extracts and fractions.

Extract /Fraction	State	IC_{50} values (µg/ml)	Regression Equation	R^2
Quercetin	26.28 (Standard Drug)		Y = 1.3233X + 15.212	0.996
Acetone	Rajasthan	436.13	Y = 0.0443X + 30.679	0.995
	Punjab	524.25	Y = 0.0423X + 27.824	0.996
	Haryana	540.75	Y = 0.045X + 25.666	0.996
Ethanol	Rajasthan	124.75	Y = 0.0408X + 44.91	0.996
	Punjab	199.38	Y = 0.0389X + 42.244	0.995
	Haryana	277.27	Y = 0.036X + 40.018	0.996
Ethyl acetate fraction	Rajasthan	59.26	Y = 0.0433X + 47.434	0.995
	Punjab	109.95	Y = 0.0429X + 45.283	0.995
	Haryana	133.62	Y = 0.0411X + 44.508	0.995

Amongst various extracts and fractions, only acetone extract, ethanol extract and ethyl acetate fraction obtained from ethanol showing presence of bioactive classes of phytoconstituents. Therefore, acetone extract, ethanol extract and ethyl acetate fraction obtained from ethanol were selected for to evaluate *in-vitro* inhibitory activity on *enzyme* lipoxigenase using well

established model.

The ethyl acetate fraction of *Citrullus colocynthis* fruits obtained from wild areas of different states Rajasthan, Punjab and Haryana exhibited strong lipoxygenase inhibition activity followed by respective ethanol extract and acetone extract, as compared to standard lipoxygenase inhibitory drug quercetin. Amongst various extracts and fractions of *Citrullus colocynthis* fruits obtained from wild areas of different states Rajasthan, Punjab and Haryana, only ethyl acetate fraction of *Citrullus colocynthis* fruits collected from Rajasthan (IC_{50} = 59.26 µg/ml) exhibited strong lipoxygenase inhibition activity followed by *Citrullus colocynthis* fruits collected from Punjab (IC_{50} = 109.95 µg/ml) and *Citrullus colocynthis* fruits collected from Haryana (IC_{50} = 133.62 µg/ml), as compared to standard lipoxygenase inhibitory drug quercetin (IC_{50} = 26.28 µg/ml). Finally, the results of the lipoxygenase inhibitory activity are depicted in tables 3 and 4.

Conclusion

The ethyl acetate fraction of *Citrullus colocynthis* fruits obtained from wild areas of different states Rajasthan, Punjab and Haryana exhibited strong lipoxygenase inhibition activity followed by respective ethanol extract and acetone extract, as compared to standard lipoxygenase inhibitory drug quercetin. The preliminary phytochemical screening showing presence of phenolic and flavonoid compounds in ethyl acetate fractions as major classes of phytoconstituents. The various scientific reports available online suggested that flavonoids and phenolic compounds such as caffeic acid, quercetin, rutin, kaempferol and apigenin (Andrade *et al*., 2019; Mbarik *et al*., 2019; Oresanya *et al*., 2020) have been used as potential lipoxigenase inhibitory agent. Therefore, finally it can be suggested that our findings of plant against lipoxigenase enzyme may be due to presence of due to presence of flavonoids and phenolic compounds. The column chromatography studies of bioactive ethyl acetate fraction were in progress to isolate bioactive lipoxigenase inhibitory constituents.

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