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Abstract:

Nutraceutical is a field of treatment which is gained more attention from people in recent times. Dietary polyphenols, as a part of nutraceutical products, constitute two groups. Hydroxycinnamic acid is one of those two groups. These acids are found naturally in various products we consume on a day-to-day basis. Coffee is one of the most common sources of hydroxycinnamic acids. Cinnamon is a spice that is consumed in different forms in various food products. The study presented investigates their capacity as anti-oxidant agents and anti-microbial agents through in-vitro and in-silico approaches. For this, commonly found infectious organisms namely, *E. coli*, and *S. aureus* were cultured. The minimum inhibitory concentration for methanolic coffee extract as well as methanolic cinnamon extract was estimated to be ~0.4 µg/ml and ~5 µg/ml. The cytotoxicity effect of these extracts was studied on cell line ie. MCF-7 (human breast cancer cell line). IC50 values for MCF-7 were calculated at 64.04 µg/ml for coffee extract, 81.12 µg/ml for cinnamon extract, and 51.14 µg/ml for standard caffeic acid. Molecular docking analysis revealed the efficiency of different hydroxycinnamic acids on protein receptors namely, PPAR γ , IL-6, TNF- α , and VEGFR. These results were supported by the tests performed on blood cultures and human blood samples obtained from the clinical partner. The present study endeavors to lay a preliminary platform to understand the efficiency and efficacy of hydroxycinnamic acid derivatives in commonly used food sources.

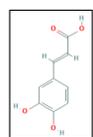
Background

- Indian nutraceutical market is expected to grow at a compounded annual growth rate of 21 percent and reach \$10 billion by the end of 2022 and \$18 by year 2025 as more people become conscious towards their health and fitness.
- Hydroxycinnamic acids are one of the most widely distributed naturally occurring phenolic acids possessing high *in-vitro* antioxidant activity.
- Data from various studies in recent decade has shown their beneficial properties.
- While all of them can be prepared synthetically, yield obtained from the process is lesser than the natural compounds.
- Extracting the compounds from food sources is cheaper, easier and yield is more stable than the ones prepared synthetically.
- Exploring this potential of hydroxycinnamic acid derivatives was the main goal of the study.

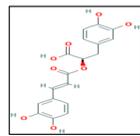
Methodology

Methanolic Extraction

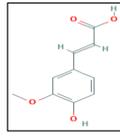
- Thin Layer Chromatography
- Estimation of Phenolic acid and caffeic acid content



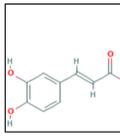
Caffeic acid
Extracted compound



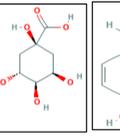
Chlorogenic acid



Cis-p-coumaric acid



Rosmarinic acid



Ferulic acid

Literature based selected compounds for in-silico studies

In-Vitro Analysis

- Anti-oxidant activity
- Anti-microbial activity
- CRP test (As the elevated level of CRP is known to elevated in active advance cancers (1) thus a reduction in the conc of the CRP level is thus indicative of anti-proliferative activity of the sample under study.

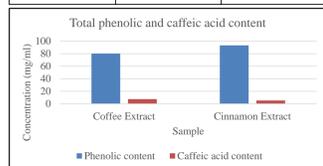
In-Silico Modelling

- Sketch and design compounds
- ADME-T properties calculation
- Molecular docking with PPAR γ , IL-6, TNF- α , and VEGF (predicted for the occurrence of numerous types of cancer and hence selected for the study) (2-6).

Results

In-vitro cytotoxicity analysis: Dose dependent response on MCF-7 cells

Sample	Phenolic content	Caffeic acid content
Coffee extract	80.11 mg/ml	7.47 mg/ml
Cinnamon extract	93.25 mg/ml	5.32 mg/ml



Total Phenolic and Caffeic acid content

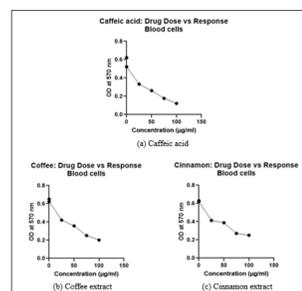
Purity check of methanolic extracts

Type of Extract	IC 50 value(ug/ml) on MCF-7
Coffee	60.04
Cinnamon	81.12
Caffeic acid	51.14

Anti-microbial Activity: Minimum Inhibitory Concentration @ µg/ml

Sample + (<i>E. coli</i>)	60	50	40	20	10	5	2.5	1.25	0.6	0.3	Sample + (<i>S. aureus</i>)	60	50	40	20	10	5	2.5	1.25	0.6	0.3	
Coffee extract	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	-	-	++
Cinnamon extract	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	-	-	++
Caffeic acid	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	-	-	++

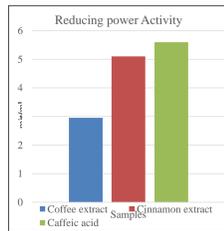
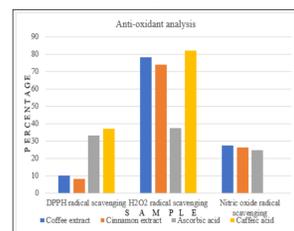
In-vitro cytotoxicity analysis



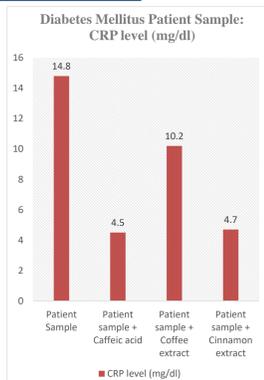
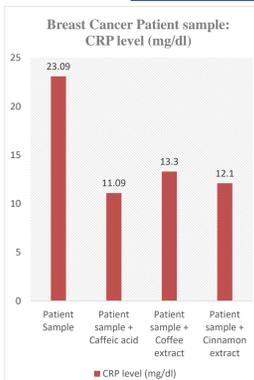
Statistical analysis of cell inhibition by the samples on blood cells or non cancerous cells shows IC50 value in the range of 45-100 µg/ml.

- Anti-microbial activity for extracts on organisms *E. coli* and *S. aureus* was estimated by microdilution method.
- MIC for coffee extract was estimated to be ~6 µg/ml while it was 4.88 µg/ml for cinnamon extract. Standard caffeic acid showed MIC to be near 5 µg/ml.

Anti-oxidant analysis



CRP level test



ADMET	Caffeic Acid	Chlorogenic Acid	Coumaric Acid	Ferulic Acid	Rosmarinic Acid
Molecular Weight	180.16	354.31	164.16	194.18	360.31
Heavy atoms	13	25	12	14	26
Aromatic heavy atoms	6	6	6	6	12
Rotatable bonds	2	5	2	3	7
H bond acceptors	4	9	3	4	8
H bond donors	3	6	2	2	5
ESOL class	very soluble	very soluble	soluble	soluble	moderately soluble
Lipinski violations	0	1	0	0	0
Bioavailability score	0.56	0.11	0.85	0.85	0.56
Synthetic accessibility	1.81	4.16	1.61	1.93	3.38

Molecular Docking – Binding Affinity Score in (Kcal/mol)

Ligand	PPAR γ	IL-6	TNF α	VEGFR-1	VEGFR-2
Caffeic acid	-2.1	-4.4	-7.2	-6.8	-7.0
Chlorogenic Acid	-1.4	-5.1	-8.6	-8.6	-7.3
Coumaric Acid	-0.8	-4.1	-7.3	-6.7	-7.0
Ferulic Acid	-1.5	-4.6	-7.3	-6.4	-7.3
Rosmarinic Acid	-1.7	-4.3	-9.4	-8.4	-8.4

Conclusion:

- From *in-vitro* cytotoxicity assay, IC50 was calculated to be 64.04 µg/ml for coffee extract and 81.12 µg/ml cinnamon extract.
- Minimum inhibitory concentration was estimated to be 5 µg/ml for coffee extract and 81.12 µg/ml for cinnamic acid.
- From biochemical tests and tests conducted with our clinical partner, it was concluded that both extracts are good anti-oxidant agents.
- From molecular docking and simulation, coffee extract and cinnamon extract were shown effective anti-oxidants.
- Coffee extract was observed to be better anti-microbial agent as well as anti-oxidant.

References:

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