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Invitro Antioxidant Property and Anticancer Property of Different Trihydroxy Flavones- An Experimental Study

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KEYWORDS

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ABSTRACT:

Classes of naturally occurring substances known as flavonoids are extensively found as secondary metabolites in the plant world are known to possess intriguing therapeutic qualities like anti-inflammatory, anti-allergic, antiviral, antibacterial, and anticancer actions. Hence this study was conducted to assess the anti cancer and anti oxidant properties of newer compounds like 3,7,3'Trihydroxy flavone, 7,3',4'Trihydroxy flavone and 6,3'4'Trihydroxy flavone.The study was approved by IEC, and conducted in the department of Pharmacology in Meenakshi medical college Hospital and Research Institute during March to July 2023. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) was performed to assess the antioxidant and cell viability assay for anticancer properties. The data were presented as Mean ± S.D and the values for IC50 were derived from the plots of the linear regression using SPSS 21 version. To evaluate mean differences at the $p < 0.05$ as significance, two-way ANOVA was utilised. Based on the DPPH, ABTS scavenging effect, percentage of inhibition of all the trihydroxy flavones investigated were found to be respectively high compared with the standards. In 24 hours MTT assay compared with control, all three compounds exhibited statistically significant difference at concentrations of 100, 150, 200, 250 and 300mg/ml and at 50mg/ml concentration the 3, 7, 3'Trihydroxy flavone alone was found to be similar to control.We conclude that all three trihydroxy flavones are effective as anti oxidant and anti cancer agents.

INTRODUCTION

Reactive oxygen species are formed both by external sources and during regular cellular aerobic respiration. Environmental variables include ultraviolet sunshine, X-ray and gamma-ray radiation, smoking, air pollution, ozone, certain medications, chemicals or pesticides, and pollutants are examples of exogenous sources [1,2]. Despite the fact that ROS are known to play beneficial roles in biological systems like the functioning of cellular signalling systems, physiological roles in cellular responses, a specific role in some pathological processes, and defence against infectious agents[3], they can still easily damage important biomolecules like nucleic acids, lipids, proteins, polyunsaturated fatty acids, and carbohydrates. Since ROS cause many human diseases, including atherosclerosis, cancer, diabetes, inflammation, cardiovascular diseases, and neurological problems[4], they are to blame for these conditions. Antioxidant defence mechanisms in living things passivate these reactive species. This defence system uses

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both enzymatic and non-enzymatic elements, as well as endogenous and foreign components, to protect cells by scavenging dangerous radicals[5].

Classes of naturally occurring substances known as flavonoids are extensively found as secondary metabolites in the plant world. They are known to possess intriguing therapeutic qualities like anti-inflammatory, anti-allergic, antiviral, antibacterial, and anticancer actions. Additionally, flavonoids are said to have antioxidant and anti-radical effects[6]. The family of polyhydroxyflavones includes well-known natural antioxidant compounds as quercetin, morin, myricetin, and kaempferol[7]. Another experiment revealed that 02"- production in the xanthine/xanthine oxidase system was occasionally inhibited by scavenger activity and enzyme inhibition. The relevance of a catechol moiety on the B ring for antioxidant and radical scavenger activity, as well as the presence of a C-7 hydroxyl substitution to promote xanthine oxidase (XO) inhibition, have been highlighted in some hypothesised structure-activity connections in this series. Because these chemicals may be helpful in the treatment of numerous disorders linked to free radical oxidations, these discoveries are important. Such substances would be particularly well suited to the pathogenesis of ischemic injury, which is characterised by an excess production of superoxide anion as a result of (i) an electron leak in the mitochondrial respiratory chain and (ii) the conversion of xanthine dehydrogenase to xanthine oxidase, which produces 02"- when converting hypoxanthine successively to xanthine and then uric acid. Therefore, substances that can inhibit xanthine oxidase and scavenge O2 may be effective as preventative measures against cellular damage during reperfusion of ischemic tissues[8]. However the literatures on antioxidant anti cancer properties of different THFs are lacking. Hence this study was conducted to assess the antioxidant and anti cancer properties of 3,7,3'Trihydroxy flavone, 7,3',4'Trihydroxy flavone and 6,3'4'Trihydroxy flavone.

MATERIALS AND METHODS

This study was approved by IEC and conducted in the Central Research Lab, Meenakshi Medical College Hospital and Research Institute during the March 2023 to July 2023.

Chemicals and reagents

The following were purchased from Sigma-Aldrich, USA: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), Xanthine, acetylthiocholine iodide, and acetylcholine enzyme (0.3U/ml). The supplier of ascorbic acid was TCI chemicals in India.Meenakshi pharmacy sold the tablet form of donezepil hydrochloride. All additional substances—including chemicals, reagents, and solvents—were of analytical quality and were bought from SRL Chemicals in India.

Procedure:

***In vitro* antioxidant activity**

DPPH Free Radical Scavenging activity assay[9]

Following previously established procedures by Koleva et al[9].,, the 2,2- diphenyl-1-picrylhydrazyl (DPPH) experiment was carried out to assess the free radical scavenging activity of the extract. 100 ml of methanol were used to make a 0.004% DPPH solution. 10 microlitres of different THFs were added to 190 litres of DPPH solution at concentrations ranging from 5 M to 160 M. The mixture was vortexed and then incubated for 20 minutes at 37 °C. The solvent in the control blank is devoid of the test substance or standard. At 517 nm, a drop in the test mixture's absorbance (caused by the quenching of DPPH free radicals) was detected. The concentration of the test mixture that caused a 50% decrease in absorbance from a control blank is known as the IC50 value. Calculating the % inhibition required repeating the experiments three times. The reference standard used was ascorbic acid. The extract's capacity to scavenge DPPH radicals was determined using the equation below.

% scavenging effect = (Absorbance of control-Absorbance of sample) 100/Absorbance of control.

ABTS radical scavenging assay[10]

Gonzalez-Palma et al[10] determined the ABTS radical scavenging activity. Water was used to dissolve 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and make it to a 7mM concentration. In order to create ABTS radical cation (ABTS+), ABTS stock solution was combined with 2.45mM potassium persulfate (final concentration) and left to stand for 12–16 hours at room temperature in the

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dark. Water was added to the ABTS+ solution to dilute it to an absorbance of 0.70 (0.02) at 734 nm. The reaction mixture contained 3mL of the ABTS radical and 0.07mL of the different THFs in concentrations ranging from 5 to 160 M. The absorbance was measured at 734 nm in a spectrophotometer after 6 minutes of incubation. The reference standard used was ascorbic acid. The following equation was used to determine the antioxidant activity.

$$\% \text{ inhibition} = [(\text{Control-Test})/\text{Control}]*100$$

$A_{control}$ = Absorbance of negative control at the moment of solution preparation

A_{sample} = Absorbance of sample after 6 min

Cell line maintenance

From the NCCS in Pune, osteosarcomacell line (MG-63) was acquired. The cells were raised in T25 culture flasks that contained DMEM with 10% FBS and 1% antibiotics as dietary supplements. Cells were kept at 37°C in a humid environment that contained 5% CO2. Confluent cells were trypsinized and passaged afterward.

Cell viability (MTT) assay[11]

By using MTT assay, the drug-treated MG-63 cancer cells' cell viability was evaluated. The assay is based on metabolically active cells converting soluble yellow tetrazolium salt into insoluble purple formazan crystals. 96-well plates with a 5x103 cell/well concentration of MG-63 cells were used. Cells were starved for three hours at 37°C in serum-free media after being washed twice with 100 microliters of serum-free medium 24 hours after plating. Following starvation, cells were exposed to various drug doses (50–300 g/ml) for 24 hours. After the course of treatment, 100 l of DMEM with MTT (0.5 mg/ml) was added to each well, along with the media from the control and drug-treated cells. The cells were then kept in a CO2 incubator for 4 hours at 37 degrees.

After that, the MTT-containing media was removed, and 1x PBS was used to wash the cells. The produced formazan crystals were then dissolved in 100 l of dimethyl sulfoxide and let to sit in the dark for an hour. The intensity of the colour created was then measured at 570 nm with a Micro ELISA plate reader. The percentage of control cells cultivated in serum-free media used to express the number of viable cells. 100% was used to indicate the cell viability in the control media without any treatment. % cell viability = [A570 nm of treated cells/A570 nm of control cells] x 100 is the formula used to calculate cell viability.

Statistical analysis

SPSS software was used to analyse the data. The data were presented as Mean and the values for IC50 were derived from the plots of the linear regression. To evaluate mean differences at the $p < 0.05$ level of significance, two-way ANOVA was utilised. Utilising the Holm-Sidak Test, the means were compared to standards groups.

RESULTS

The results of the cell viability analysis for 3, 7, 3'Trihydroxy flavone, 6, 3', 4' Trihydroxy flavone, and 7, 3', 4' Trihydroxy flavone at various concentrations are presented in Table 1. The standard values, mean, and standard error of the mean (SEM) for each concentration were assessed. Notably, all three trihydroxy flavones exhibited dose-dependent effects, with an increase in cell viability observed as the concentration increased. Specifically, at the highest concentration of 160 µg/ml, 3, 7, 3'Trihydroxy flavone showed a mean test value of 73.29, 6, 3', 4' Trihydroxy flavone exhibited a value of 71.42, and 7, 3', 4' Trihydroxy flavone demonstrated a value of 79.49. These findings suggest a potential dose-dependent positive impact on cell viability for the investigated trihydroxy flavones. The significance of these results lies in the potential application of these compounds as agents promoting cell viability, which is crucial in the context of anti-cancer research and therapeutic development(Table-1).

Table 1: Cell Viability Analysis of Trihydroxy Flavones at Various Concentrations

Concentration (µg/ml)	Standard	SEM (Mean)	Test value	SEM (Sample)
3, 7, 3'Trihydroxy flavone				
10	24.37	5.9	17.29	2.3
20	38.28	4.6	28.39	4.3

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40	57.29	4.5	45.29	5.5
80	71.29	4.4	61.29	6.1
160	89.29	7.4	73.29	8.2
6, 3', 4' Trihydroxy flavone				
10	30.29	4.29	25.39	3.29
20	42.3	3.53	37.20	4.39
40	57.39	6.2	51.29	5.48
80	68.29	7.59	59.10	6.39
160	78.29	8.29	71.42	7.39
7, 3', 4' Trihydroxy flavone				
10	34.28	3.29	31.39	5.38
20	47.29	3.84	41.94	4.39
40	54.29	4.39	52.85	3.84
80	67.32	6.3	61.39	6.39
160	85.39	5.38	79.49	7.39

DPPH radical scavenging activities of various concentrations of different THFs. DPPH assay was performed in triplicate and the mean ± SD were calculated with *p < 0.05. Ascorbic acid was used as a standard.

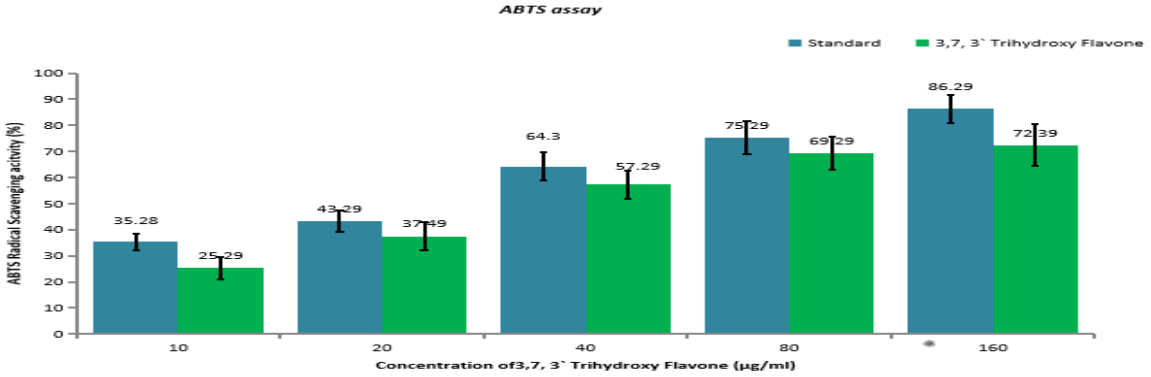

Figure 1: ABTS cation radical scavenging activities of various concentrations of 3,7,3'.ABTS assay was performed in triplicate and the mean ± SD were calculated with *p < 0.05. Ascorbic acid was used as a standard.

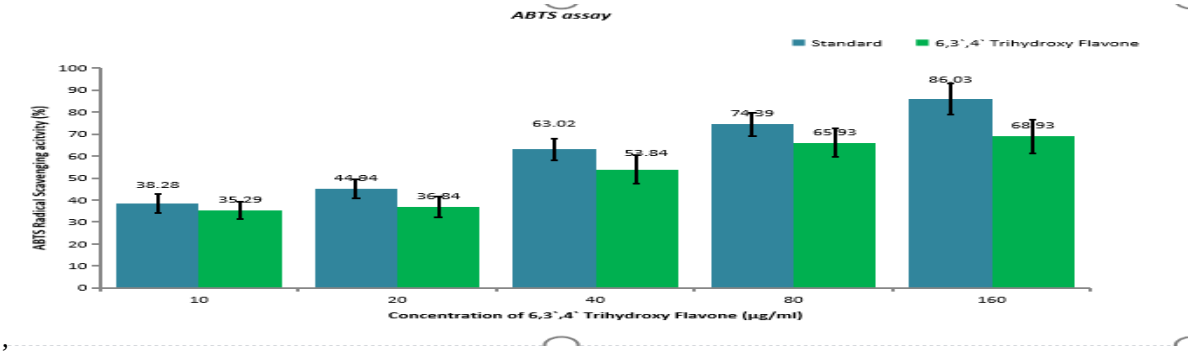

Figure 2: ABTS cation radical scavenging activities of various concentrations of 6,3'4' .ABTS assay were performed in triplicate and the mean ± SD were calculated with *p < 0.05. Ascorbic acid was used as a standard.

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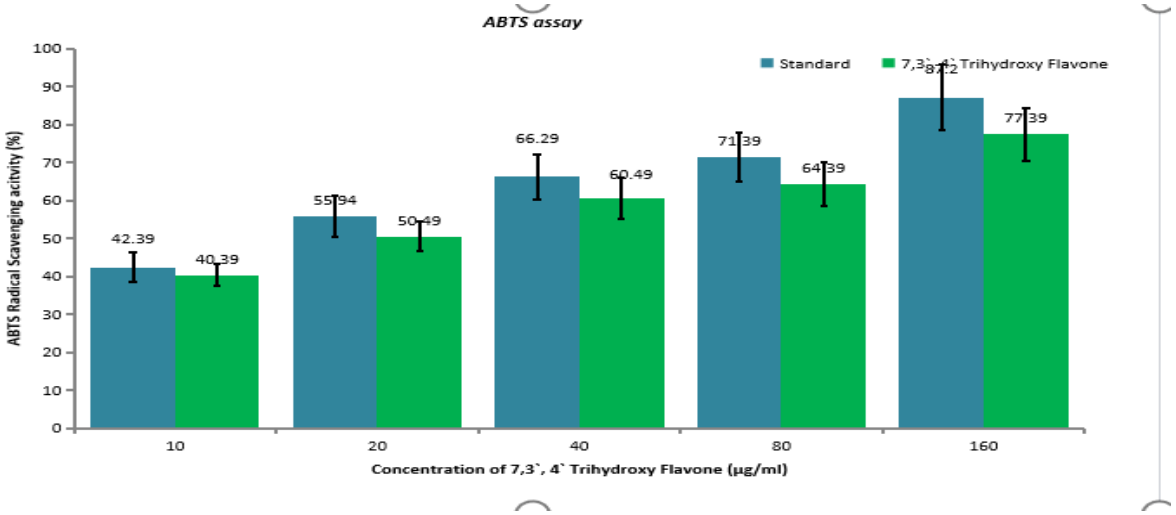

Figure 3: ABTS cation radical scavenging activities of various concentrations of .AB7,3',4'TS assay were performed in triplicate and the mean ± SD were calculated with *p < 0.05. Ascorbic acid was used as a standard.

Based on 24 hours MTT assay compared with control 3, 7, 3'Trihydroxy flavones was found to show statistically significant difference at concentrations of 100, 150, 200, 250 and 300 however not at the concentration of 50. Similarly, compared with control 6, 3',4'Trihydroxy flavones and 7, 3', 4' Trihydroxy flavone were found to show statistically significant difference at concentrations of 50, 100, 150, 200, 250 and 300.(Table 2)

Table 2 illustrates the impact of different concentrations of 3, 7, 3'Trihydroxy flavone, 6, 3', 4' Trihydroxy flavone, and 7, 3', 4' Trihydroxy flavone on cell viability, as assessed through the MTT assay over a 24-hour period. Notably, all three compounds demonstrated a concentration-dependent decrease in optical density (O.D), indicating a decrease in cell viability compared to the control group. Percentage inhibition showed a consistent increase with higher concentrations, supporting the dose-dependent effect. The mean values further reinforced this trend, with statistical significance (*p < 0.05) observed at various concentrations for each compound. This emphasizes the potential cytotoxic impact of these trihydroxy flavones, making them promising candidates for further investigation in anti-cancer research and therapeutic development. The significant p-values underscore the importance of these findings, suggesting a meaningful influence on cell viability that warrants further exploration.

Table 2: Cell Viability (MTT assay) Impact of Various Concentrations in 24 hours

Parameter	Control	50	100	150	200	250	300
3, 7, 3'Trihydroxy flavone							
O.D	2.38	1.78	1.47	1.17	0.88	0.41	0.46
O.D	2.00	1.95	1.57	1.36	1.36	0.73	0.48
O.D	2.32	1.76	1.28	1.17	0.97	0.68	0.68
%	100.00	74.65	61.52	49.10	37.05	17.37	19.47
%	100.00	97.50	78.39	67.66	68.01	36.43	24.00
%	100.00	75.94	55.20	50.41	41.87	29.37	29.41
Mean	100.00	82.70	65.04	55.72	48.98	27.72	24.29
SE	0.00	10.48	9.79	8.46	13.60	7.87	4.06
p value	-	0.0753	0.0249*	0.0151*	0.024*	0.0074*	0.0023*

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6, 3', 4' Trihydroxy flavone							
O.D	3.341	2.07	2.11	1.77	0.94	0.32	0.33
O.D	3.507	2.65	1.94	1.42	0.84	0.49	0.44
O.D	3.183	2.54	1.78	1.85	0.78	0.59	0.27
%	100	62.08	63.12	53.07	28.08	9.70	9.79
%	100	75.53	55.29	40.52	23.87	14.03	12.52
%	100	79.89	56.05	58.09	24.60	18.63	8.61
Mean	100	72.50	58.15	50.56	25.51	14.12	10.30
SE	0	7.58	3.53	7.39	1.84	3.65	1.64
p value	-	0.0187*	0.0023*	0.0086*	0.0006*	0.0012*	0.0001*
7, 3', 4' Trihydroxy flavone							
O.D	2.84	1.97	1.86	1.293	0.96	0.386	0.26
O.D	2.50	1.63	1.49	1.538	0.92	0.567	0.18
O.D	2.69	1.98	1.59	1.303	0.73	0.473	0.38
%	100	69.25	65.42	45.38	33.90	13.54	9.19
%	100	65.44	59.76	61.44	36.83	22.65	7.31
%	100	73.60	59.13	48.36	27.43	17.55	14.17
Mean	100	69.43	61.44	51.73	32.72	17.91	10.22
SE	0	3.33	2.82	6.97	3.92	3.72	2.89
p value	-	0.0021*	0.0005*	0.0088*	0.0019*	0.0023*	0.0007*
***Significant**							

DISCUSSION

For more than a century, flavonoids have been recognised as a significant plant product. In 1936, Rusznyak and Szent Gyorgyi published papers for the first time about their biological activity[12]. Although "vitamin P" was initially proposed as a name for flavonoids, it was eventually abandoned[13]. Flavonoids, which are made up of subclasses including flavones, flavanones, flavanols, isoflavones, anthocyanidins, and flavanoids, are a vast group of polyphenolic substances that are commonly present in all foods of plant origin[14]. However, there is still disagreement over the various falvones' antioxidant characteristics, such as those of 3,7,3'Trihydroxy flavone, 7,3',4'Trihydroxy flavone, and 6,3'4'Trihydroxy flavone.

In a study, Fraga CG et al[15]found that giving mice eriodyctiol and (+)-catechin prevented the CCI-induced increase in sifu hepatic chemiluminescence by 32 and 38%, respectively. 3,4 Cynarin showed no impact, and diaffeoylquinic acid was less effective (13%). These substances, along with other polyphenols, have previously been tested for their capacity to prevent the chemiluminescence of mouse liver homogenates that is caused by tert-butyl hydroperoxide. These flavonoids and polyphenols may have antioxidant properties that contribute to their in vitro and in vivo effects, making them intriguing compounds to be researched as water-soluble inhibitors of lipid peroxidation and other free radical-mediated cell damage.

According to Cotelle N. et al[16] the 2',3 ',4'-OH substitution on the B ring is essential for both the MDA test's ability to block peroxidation of tissue lipids and the DPPH assay's ability to detect radical scavenger activity. ESR has investigated how these kinds of chemicals produce stable radicals. Additionally, it has been discovered that 7-hydroxy-flavones are strong xanthine oxidase competitive inhibitors. It has been suggested that the C-7 OH of flavones could replace the C-2 or C-6 OH of xanthine in the enzyme's active site. The 7-hydroxy flavones' C-4' OH or C-4' OMe substitutions are unfavourable for an active site fit. The exact mechanism by which the 2',3 ',4'-trihydroxy-flavones suppressed XO is still unknown. In conclusion, our research shows that hydroxy-flavones have intriguing antioxidant capabilities, which may be seen in their ability to either scavenge free radicals (in the case of 2', 3', and 4' trihydroxy-flavones) or to competitively inhibit xanthine oxidase (in the case of 7-hydroxy-flavones). These substances might be used as therapeutic candidates to treat diseases brought on by free radical oxidation.

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Nine different flavonoid aglycons, including quercetin, kaempferol, myricetin, apigenin, luteolin, daidzein, genistein, formononetin, and biochanin A, were the subjects of an investigation by Lugasi A. et al[17]. On their in vitro antioxidant capabilities. The chemical structure of flavonoids largely determines their in vitro antioxidant capacity. Using an RP-HPLC technique, the amount of such flavonoids in commonly consumed fruits and vegetables in Hungary was assessed. Regarding the total amount of flavonoids, abundant sources included lentils, onions, parsnips, spinach, various celery parts, and parsnips. Berries were a particularly good source of flavonoids among the fruits. Two groups were used to estimate the intake of flavonoids: the first comprised more than 500 schoolchildren and young women between the ages of 12 and 15; the second, roughly 200 healthy adults between the ages of 25 and 60. The children and adults consumed 19.5–26.6 and 18.8–28.9 mg/day of dietary flavonoids on average, respectively. The subjects' consumption of flavonoids revealed significant disparities. Flavonoids were consumed by the children's group in amounts ranging from 0 to 179 mg/day, and by the adults' group in amounts ranging from 0.5 to 310 mg/day. Although just two of the study's groups accurately represented the entire Hungarian population, it is assumed that the population's average daily flavonoid intake is similar to these results. The estimated Hungarian intake is quite comparable to other intakes reported in the literature.

According to Chen Si et al[18] the proportion of 30% and 75% ethanol elution fractions (EEFs) was 26.20% and 62.57%, respectively. The DPPH scavenging activity of 75% and 30% EEFs was 95.51% and 78.85%, respectively. 5,3′,4′-trihydroxyflavone and hyperoside both showed significant scavenging abilities, with peak areas reduced by 82.69% and 76.04%, respectively. When compared to the normal control group (NC) fed a basal diet, the levels of the three treated groups' superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), and total antioxidant capacity (T-AOC) significantly improved by 3.89-24.49%, 0.53-7.39%, and 0.79-11.79%, respectively. Malondialdehyde (MDA) reduced by 0.47–18.27%, meanwhile. The feed to gain ratio of the three treated groups decreased by 2.98 to 16.53% as compared to the NC, and the broiler survival rate dramatically improved. Because of their high antioxidant properties, 75% of the lucerne EEFs that were isolated showed promise as a feed supplement for poultry and animals.

Kwon SH et al[19] looked into the ways that hydrogen peroxide (H_2O_2)-related neurotoxin 3′,4′,7-trihydroxyflavone (THF) protects neuronal cells from neuronal cell death. According to their findings, THF protects neuronal cells from H_2O_2induced oxidative stress, possibly via reducing ROS, protecting mitochondria, and modulating NF-B via MAPK and PI3K/Akt pathways. THF is a prospective contender as a treatment for neurodegenerative illnesses due to its neuroprotective properties.

In the CAA assay, Wolfe KL et al[20] identified structure-activity correlations of certain flavonoids. In the CAA assay, flavonoids containing a 3′,4′-o-dihydroxyl group in the B-ring, a 2,3-double bond paired with a 4-keto group in the C-ring, and a 3-hydroxyl group all shown the highest antioxidant activity. Isoflavones lacked any antioxidant action in cells. Galloyl-modified flavanols displayed greater antioxidant activity than those without it, and the addition of a B-ring 3′,4′,5′-trihydroxyl group increased their potency even more. The flavonoids' ORAC and CAA levels did not correlate. It may be useful to understand the structure-activity connections in the CAA assay when evaluating flavonoids' potential in vivo antioxidant activity.

In addition to demonstrating the hemorheological potentials of flavonoids that have specific protein-antioxidant activities, Bilto YY et al[21]. reported on the significance of the chemical groups substituted on the basic skeleton of flavonoids in determining the kind of antioxidant activity.

The antioxidant and anticancer properties of the flavonoid chemicals found in Plectranthus amboinicus Spreng were identified by Manurung K et al[22]. They claimed that the ethanolic extract of Plectranthus amboinicus Spreng leaves contains a wide range of flavonoid chemicals that all have extremely potent anti-cancer and antioxidant activities. Strong antioxidant activity can be found in spreng leaves. In complicated biological systems where it can interact with other antioxidants like vitamins, luteolin, a flavone, exhibits good radical scavenging and cytoprotective effects, according to research by Seelinger G et al[23]. At micromolar concentrations, luteolin exhibits particular anti-inflammatory actions that are only partially explained by its antioxidant properties. Antioxidative enzymes are activated,

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the NF-B pathway is suppressed, and pro-inflammatory chemicals are inhibited as part of the anti-inflammatory function. After parenteral and oral administration, luteolin proved efficacious in animal models of inflammation and decreased enhanced vascular permeability. Even though luteolin makes only a small portion of our diet, epidemiological research suggests that it may be able to prevent diseases like cardiovascular disease that are linked to inflammatory processes. Luteolin frequently exists in plants as glycosides, however following nutritional intake, they are cleaved and the aglycones are conjugated and metabolised, which must be taken into account when evaluating in vitro investigations.

CONCLUSION

In the present study we conclude that based on DPPH and ABTS scavenging effect, percentage of inhibition, respectively were found to be high compared with the standards. Based on 24 hours MTT assay compared with control all three compounds are effective as anti cancer agents at concentrations of 100mg/ml and above however 3, 7, 3'Trihydroxy flavones was not effective at concentration of 50mg/ml. Further in depth analysis of these compounds are needed including the molecular studies of the compounds before starting clinical trials.

Declarations

Author's Contributions

G. Naga Phaneesh carried out the whole research work under the guidance of

Dr. K. Parimala - helping in the designing of the study.

Dr.S.Shanmuga Priyan -helping in the manuscript writing.

Dr. N. Muninathan - helping in the laboratory research work.

Dr. D.Ganesh kumar - helping in the manuscript correction.

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Conflict of interest: None declared

Ethical approval: This study was registered with Institutional Ethical Committee.

ABBREVIATIONS

THF -Trihydroxy flavone

DPPHassay - 2,2-diphenyl-1-picrylhydrazyl assay

ABTSassay -2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)assay

MTT assay: 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide Assay,

REFERENCES

[1] Yanai N, Shiotani S, Hagiwara S, Nabetani H, Nakajima M. Antioxidant combination inhibits reactive oxygen species mediated damage. Bioscience, biotechnology, and biochemistry. 2008;72(12):3100-6.

[2] Sen S, Chakraborty R, Sridhar C, Reddy YS, De B. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. Int J Pharm Sci Rev Res. 2010 ;3(1):91-100.

[3] Gülcin I. Antioxidant activity of food constituents: an overview. Archives of toxicology. 2012 ;86:345-91.

[4] Grune T, Shringarpure R, Sitte N, Davies K. Age-related changes in protein oxidation and proteolysis in mammalian cells. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2001 ;56(11):B459-67.

[5] Matés JM, Pérez-Gómez C, De Castro IN. Antioxidant enzymes and human diseases. Clinical biochemistry. 1999 ;32(8):595-603.

[6] Bors, W.; Heller, W.; Michel, C.; Saran, M. Flavonoids as antioxidant: Determination of antiradical efficiencies. *Methods Enzymol.*; 1990; 186:343-355.

[7] Cotelle, N.; Bernier, J.-L.; Htnichart, J.-P.; Catteau, J.-P.; Gaydou, E.; Wallet, J.-C. Scavenger and antioxidant properties of ten flavones. *Free Radic. BioL Med.*1992;

[8] 13:211-219.

[9] Werns S. W.; Lucchesi R. Free radicals and ischemic tissue injury. *TIPS.*1990;11:161-166.

[10] Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal. 2002 ;13(1):8-17.

[11] González-Palma I, Escalona-Buendía HB, Ponce-Alquicira E, Téllez-Téllez M, Gupta VK, Daze-GodínezG and Soriano-Santos J (2016) Evaluation

of the Antioxidant Activity of Aqueous and Methanol Extracts of Pleurotusostreatus in Different Growth Stages. Front. Microbiol. 7:1099.

[12] Abida P, De Britto AJ, Antoney J, Stephan Raj TL . Evaluation of in vitro anticancer activity of Symplocos cochinchinensis(Lour.) S. Moore bark. Int J Herb Med.2016;(4):117–119 .

[13] Rusznyak, S.P.; Szent-Gyorgyi, A. Vitamin P as Flavonoids. Nature 1936;138: 27.

[14] Kuo, S.M. Dietary Flavonoid and Cancer Prevention: Evidence and Potential Mechanism. Critical Reviews in Oncogenesis. 1997; 8: 47–69.

[15] Patel, D.; Shukla, S.; Gupta, S. Apigenin and Cancer Chemoprevention: Progress, Potential and Promise (Review). International Journal Oncology 2007; 30:233–246.

[16] Fraga CG, Martino VS, Ferraro GE, Coussio JD, Boveris A. Flavonoids as antioxidants evaluated by in vitro and in situ liver chemiluminescence. Biochemical pharmacology. 1987 ;36(5):717-20.

[17] Cotelle N, Bernier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM. Antioxidant properties of hydroxy-flavones. Free radical biology and Medicine. 1996 J;20(1):35-43.

[18] Lugasi A. The role of antioxidant phytonutrients in the prevention of diseases. Acta biologica szegediensis. 2003 ;47(1-4):119-25.

[19] Chen S, Li X, Liu X, Wang N, An Q, Ye XM, Zhao ZT, Zhao M, Han Y, Ouyang KH, Wang WJ. Investigation of chemical composition, antioxidant activity, and the effects of alfalfa flavonoids on growth performance. Oxidative medicine and cellular longevity. 2020 ;2020.

[20] Kwon SH, Hong SI, Ma SX, Lee SY, Jang CG. 3′, 4′, 7-Trihydroxyflavone prevents apoptotic cell death in neuronal cells from hydrogen peroxide-induced oxidative stress. Food and Chemical Toxicology. 2015 ;80:41-51.

[21] Wolfe KL, Liu RH. Structure− activity relationships of flavonoids in the cellular antioxidant activity assay. Journal of agricultural and food chemistry. 2008 ;56(18):8404-11.

[22] Yousif Y B, Sanaa S, Talal A, Shtywy A. Structure-activity relationships regarding the antioxidant effects of the flavonoids on human erythrocytes. Natural Science. 2012 .

[23] Manurung K, Sulastri D, Zubir N, Ilyas S. In silico anticancer activity and in vitro antioxidant of flavonoids in Plectranthus amboinicus. Pharmacognosy Journal. 2020;12(6s).

[24] Seelinger G, Merfort I, Schempp CM. Anti-oxidant, anti-inflammatory and anti-allergic activities of luteolin. Planta medica. 2008 ;74(14):1667-77.