Formulation and Evaluation of Nutraceutical using herbal Extract

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Abstract -Oxidation is a necessary component of aerobic exercise throughout life. It is a chemical reaction in which electrons or hydrogen are transferred from a substance to an oxidizing agent, resulting in the production of free radicals. These highly reactive free radicals trigger a chain reaction that causes cellular damage. The development of cancer has been linked to the creation of free radicals that surpass the body's antioxidant capacity, resulting in oxidative stress. As a result, researchers have turned to plants, which contain antioxidants that can scavenge and disrupt free radicals' harmful effects. Plants produce a wide range of molecules known as phytochemicals that have significant antioxidant effects and have been proven to be effective in the treatment of a variety of ailments. This study focuses on the antioxidant capacity of plants with therapeutic properties, which can benefit individuals, businesses, and healthcare facilities.

Keyword : Antioxidant activity, phytochemical, Medicinal plant, free radical.

INTRODUCTION

Cancer is the second leading cause of death globally, accounting Cancer is a large group of diseases, which affects millions of people worldwide [1]. Cancer is the abnormal growth and proliferation of cell or tissue [2]. In past decades, anticancer research has led to remarkable results despite many of the approved drugs still being characterized by high systemic toxicity mainly due to the lack of tumour selectivity, over production of free radical due to various drugs and present pharmacokinetic drawbacks, including low water solubility, that negatively affect the drug circulation time for an estimated 9.6 million deaths, or 1 in 6 deaths, in 2018. Lung, prostate, colorectal, stomach and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, cervical and thyroid cancer are the most common among women [4]. Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in year 2020. The most common in year 2020 (in terms of new cases of cancer) were breast 2.26 million cases, lung 2.21 million cases, colon and rectum 1.93 million cases, prostate 1.41 million cases. The most common causes of cancer death in year 2020 were: lung (1.80 million deaths) colon and rectum (916 000 deaths), liver (830 000 deaths),stomach (769 000 deaths),and breast (685 000 deaths. Despite advances in treatment, current anticancer drugs often have limited effectiveness, lack of cancer selectivity, serious side effects and drug resistance in cancer cells[2].

Production of free radicals in the human body

Free radicals and other ROS are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals[8]. Free radical formation occurs continuously in the cells as a consequence of both enzymatic and nonenzymatic reactions. Enzymatic reactions, which serve as source of free radicals, include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis, and in the cytochrome P-450 system[9]. Free radicals can also be formed in nonenzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing reactions. Some internally generated sources of free radicals are[10].

- Mitochondria
- Xanthine oxidase
- Peroxisomes
- Inflammation
- Phagocytosis
- Arachidonate pathways
- Exercise
- Ischemia/reperfusion injury

Some externally generated sources of free radicals are:

- Cigarette smoke
- Environmental pollutants
- Radiation
- Certain drugs, pesticides
- Industrial solvents
- Ozone

Concept of oxidative stress

The term is used to describe the condition of oxidative damage resulting when the critical balance between free radical generation and antioxidant defenses is unfavorable.[11] Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids.[12] Short-term oxidative stress may occur in tissues injured by trauma, infection, heat injury, hypertoxia, toxins, and excessive exercise. These injured tissues produce increased radical generating enzymes (e.g., xanthine oxidase, lipogenase, cyclooxygenase) activation of phagocytes, release of free iron, copper ions, or a disruption of the electron transport chains of oxidative phosphorylation, producing excess ROS. The initiation, promotion, and progression of cancer, as well as the side-effects of radiation and chemotherapy, have been linked to the imbalance between ROS and the antioxidant defense system. ROS have been implicated in the induction and complications of diabetes mellitus, age-related eye disease, and neurodegenerative diseases such as Parkinson's disease.[13] To combat the toxic effects of reactive oxygen species (ROS), cells have developed multiple defense strategies that include (a) production of enzymes consisting of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxides (GPx), and (b) synthesis of non-enzymatic reducing agents including glutathione and vitamins E and C. Failure to produce these antioxidant defense molecules on time by cells may result in disease development. Hence dietary supplementation with downloaded from naturally occurring antioxidants helps in preventing ROS-induced diseases, particularly diabetes and cancer[14]. The overproduction of free radicals is known to cause several chronic diseases including cancer. However, increased production of free radicals by chemotherapeutic drugs is also associated with apoptosis in cancer cells, indicating the dual nature of free radicals. Among various natural compounds[15] A balance between free radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's *ability* to regulate them, a condition known as oxidative stress ensues. Free radicals thus adversely alter lipids, proteins, and DNA and trigger a number of human diseases. Hence application of external source of antioxidants can assist in coping this oxidative stress. Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have recently been reported to be toxic for human health. Thus, the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years. Flavonoids are plant-derived phenolic compounds that reduce ROS in cells.[16]. The ability of flavonoids to reduce ROS is primarily due to the presence of 3hydroxypyran-4-one, 5- o, and 4-carbonyl groups in the C ring, rather than the 3,4-dihydroxy group in the B ring [17]. Flavonoids exhibit potent antiinflammatory, antiviral, anti-allergic, antimicrobial, and anticarcinogenic activities[16]. insulinomimetic flavonoid found in citrus fruits.

MATERIALS AND METHODS

Naringenin API Extract from orange fruit peels 2 Mannitol Bulking agent Central Drug House (P) Ltd. 3 Microcrystalline cellulose Blinder Research-Lab Fine Chem Industries, Mumbai 4 Dried starch Filler agent Central Drug House (P) Ltd. 5 Povidone K30 Blinder Central Drug House (P) Ltd. 6 Magnesium stearate Lubricant Pallav Chemicals &Solvent Pvt. Ltd. 7 Ethanol Solvent Variety traders, Amravati 8 Choroform Solvent Variety traders, Amravati

Methodology

Collection and Authentification of fruit plant Fresh oranges fruits were collected from the local farm house Tiosa, Amravati. The plant was identified and authenticated by Dr. Ganesh B. Hedawoo of the Department of Botany, Shri Shivaji Science College, Amravati The albedo (Inner white spongy part) from the oranges peels was separated from the of the peels which is flavedo (oranges exterior). albedo was then air dried for 4-5 days. And dried peels were powdered in grinder for direct extraction.

Extraction of naringin

The extraction of naringin from the albedos of grapefruit was carried out The extraction process was carried out by following method to get a better yield of naringin using both dry albedo.

Method:

Soxhlet extraction using dry albedo in methanol: The extraction was carried out using the Soxhlet apparatus with 40g of the dried albedo of Orange fruit (figure7). The solvent used for Soxhlet extraction was methanol and temperature was maintained at 55-65 ℃ for approximately 3 h. After leaving the extract overnight, colourless crystals of pectin were collected from the extract and the remaining total methanolic extract was reduced to 10-12 g under reduced pressure at 45-50 ℃ and 20-25 cm3 of water added to that extract. After stirring for 30 min at 60-70 ℃, this methanolic extract was left for 3 days in a stoppered flask after adding 3 cm3 of chloroform in it. On standing for 3 days, fine naringin crystals were formed and were filtered out using sintered crucible and collected.

Hydrolysis of Naringin

Naringenin is derived from the hydrolysis of naringin. Simple and efficient hydrolysis of naringin carried out to get a better yield.

Method

Acidic Hydrolysis in Ethanol. 2% 15 cm3 H_2SO_4 ethanolic solution was added to 0.1 g of naringin in the round bottom flask. The mixture was then stirred and refluxed at 80 ℃ to 85 ℃ for 13h.

Preliminary Phytochemical Screening

Test for carbohydrates (Benedict's test)

In 2ml of extract add 2ml of Benedict's reagent and boiled. Formation of orange red precipitate indicates the presence of carbohydrates [76]

Flavanoids (alkaline reagent test)

2ml of 2% sodium hydroxide solution was added to 2ml of the extract. Appearance of yellow colour precipitation indicates the presence of flavonoids [77, 78,79]

Test for Triterpenoids (Salkowski's test)

In 2ml of extract add 1ml of chloroform followed by a few drops of concentrated H_2SO_4 on the side of the test tube and shaken well, formation of yellow colour at the lower layered indicates the presence of triterpenoids [80]

Test for Saponins (frothing test)

2ml of extract was diluted with 10ml of distilled water in a test tube and shaken for 5 mins, observation of stable foam indicates the presence of saponins [81].

Test for tannins (ferric chloride test)

2ml of the extract was mixed with few drops of 10% ferric chloride solution, the change of colour into dark blue or green indicates the presence of gallic tannins and catechol tannins [82]

Test for glycosides (Keller-Kilani test)

A mixture of 4ml of glacial acetic acid and 1 drop of 2.0% $FeCl_3$was added to 10ml of the extract followed by the addition of 1ml of concentrated H_2SO_4. Formation of brown ring between the layers indicates the presence of cardiac glycosides [83].

Thin Layer Chromatography

Preparation of TLC plates Weighed 30 grams of silica gel "G," it was suspended homogeneously in 60 milliliters of distilled water for a duration of two minutes. The suspension was applied to the glass plate, which was allowed to air dry until the layer's transparency vanished. After 30 minutes of drying at 110°C in a hot air oven, the plates were placed in a dry environment and utilized as needed.

Preparation of Sample Solution

Samples are first dissolved in an appropriate solvent before being applied, typically in amounts of 1–10 µl, to the beginnings of a TLC sheet or plate. The formula for Finding the Rf value Rf is equal to (solute travel distance / solvent travel distance). Using the substance mixture to achieve separation. The various samples' solutions were collected in capillary tubes and spotted on a TLC plate two centimeters above the bottom.

Development of the chromatogram

Following the sample application on the adsorbent, the TLC plate was placed in the solvent within the TLC glass chamber, allowing the mobile phase to pass through the adsorbent phase to a depth of approximately 3/4 of the plate. The separation occurred, and following spaying with the appropriate

detecting agents, the colored dots were obtained. The images of the few TLC plates and the tabulated results of the determination of the Rf values for the various places are included in the table.

Organoleptic Properties

Organoleptic properties of naringenin were determined by various standard method. The color, odour, taste, and state of herbal drug were characterized and recorded using descriptive terminology.

Colour: off yellow

Odour: Charecteristic

State: Solid (Powder form)

Powder Characterization

Physical parameter of bulk drug like Angle of repose, bulk density, tapped density (BD and TD), carr's index and hausner's ratio was measured.

Angle of Repose

Angle of repose (θ) is the maximum angle between the surface of a pile of powder and horizontal plane. It is usually determined by Fixed Funnel Method and is the measure of the flowability of powder. The accurately weighed powder was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the powder. The powder was allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured and the angle of repose was calculated using the following equation. $\tan(\theta) = h/r$ Therefore; $(\theta) = \tan^{-1}(h/r)$ Where, h= Height of pile, r= Radius of the base of the pile (θ)= Angle of repose

Density

Powder density may influence compressibility, tablet porosity, dissolution and other properties

Bulk Density (BD)

Bulk Density (BD) Largely depends on particle shape. As the particle become more spherical in shape, bulk density decreases. The smaller granules are able to form close, more intimate packing than larger granules. The term bulk density refers to measure used to describe a packing or granules. It is the weight the volume ratio of the substance expressed in gm/cm3 and calculated by using the following equation Bd=W/V1 Where, Bd= Bulk density, W= Weight of sample in gram, VI= Initial volume of sample in cm3

Tapped Density (TD)

It is a limiting density attained after "tapping down," usually in a device that lifts and drops a volumetric measuring cylinder containing the powder from a fixed distance.The tapped density is the weight by tapped volume ratio expressed in gm/cm3 and calculated by using the following equation. Td=W/VT Where, Td= Tapped density, W= Weight of sample in gram,VT= Final tapped volume of sample in cm3

Carr's Compressibility Index

Compressibility is the ability of powder to decrease in volume under pressure. Compressibility is a measure that is obtained from density determinations. The compressibility index of the powder was determined by Carr's compressibility index Carr's Index =[(TD-BD) ×100/ TD]

Hausner's Ratio

Hausner's found that the ratio tapped density/bulk density was related to inter particle friction as such, and could be used to predict powder flow properties. Hausner's Ratio = Tapped Density / Bulk Density.

Formulation Development

Tablet formulation

Preparation of Granules

Naringenin were used to prepared granules by wet granulation technique with the formula shown in

Table No: 1 Formulation for tablet

Sr no	Content	F1	F2	F3
1	Naringenin	40.00	40.00	40.00
2	Mannitol	148.00	152.00	146.00
3	Microcrystallinecellulose	58.00	54	60.00
4	Dried starch	1.87	1.87	1.87
5	Povidone K30	0.25	0.25	0.25

6	Magnesiumstearate	1.87	1.87	1.87
Total weight (mg)		250	250	250

The accurately weighed quantities of naringenin were mixed mixed with mannitol, Dried starch, Microcrystalline cellulose and then passed through sieve no 30

- Weigh quantity of Povidone k 30 and measure quantity of water mixed and slurry was prepared
- Mixed with above powder of API and excipients with sufficient quantity of slurry prepared to obtain dough mass.
- The wet mass was passed through sieve no 10 □ Granules were dried in a hot air oven at 50 to 55°C
- Dried granules were passed through sieve no 30
- The granules were lubricated by magnesium stearate

Compression of Granules into Tablet
Compressed the blend after lubricating the granules into tablet by using tablet compression machine.

Evaluation of tablet
Physical Appearance
The general appearance of a tablet, its identity and general elegance is essential for consumer acceptance, for control of lot-to-lot uniformity and tablet-to-tablet uniformity. The control of general appearance involves the measurement of size, shape, colour, presence or absence of odour, taste etc.

Thickness:
The crown thickness of tablets is measured using a micrometer to determine density and ensure uniformity. Variations in thickness and diameter impact hardness and dissolution. Tablet diameter and thickness are measured with a vernier caliper, with the least count calculated by dividing the smallest division on the main scale by the total divisions on the vernier scale. Place the tablet between the jaws, record the main and vernier scale readings, and repeat for ten tablets. Calculate the average thickness and range to ensure individual tablet thickness falls within it.

Hardness:
Hardness affects the tablet's ability to resist chipping, abrasion, and breakage during storage, transportation, and handling. Adjustments in hardness ensure proper pressure on the tabletting machine. If too hard, tablets may not disintegrate or meet dissolution specs; if too soft, they won't withstand subsequent processing. To test, place the tablet between fixed and moving anvils of the Monsanto hardness tester, adjust the scale to zero, apply pressure until the tablet breaks, and record the reading. Repeat for each tablet .The hardness was measured in terms of Kp.

Weight variation:
Randomly 20 tablets were selected after compression and the average weight was determined. None of tablets deviated from the average weight by more than + 4.5%. the weight values were expressed in milligrams. This is an important parameter in process quality control test to be checked frequently. Correction were made during the compression of tablets. Any variation in weight of tablets leads to either under medication in overdose. So every tablet in each batch should have a uniform weight.Weight variation specification.

Friability
Friability is the measure of tablet strength. friabilator having model no FT 1020 of LAB INDIA was used for testing the friabiity using following procedure. Ten tablets were weighed accurately and placed in the tumbling apparatus that revolves at 25 rpm dropping the tablets through a distance of six inches with each revoution. After 4 min, the tablets were weighed and the percentage loss in the tablet weight was determined.
% Friability= Initial weight- Final Weight/ Initial Weight x100……

Dissolution studies
The paddle method of the USP dissolution test apparatus was used to calculate the in vitro release of Naringenin tablet. At 370 0.50c and 100 rpm, the dissolving test was conducted using 900ml of water were taken out of the dissolving equipment and replaced with a new dissolution medium. A UV visible spectrophotometer was used to test the samples' absorbance at 292 nm after they had been filtered. To

calculate the release profile, the cumulative percentage of drug release was plotted against time.

Disintegration Test

Place one tablet in each of the six tubes of the basket. Add a disc to each tube and run the apparatus using pH1.2 maintained at 37±2 °C as the immersion liquid. The assembly should be raised and lowered between 30 cycles per minute in the pH 1.2 maintained at 37±2 °C. The time in the second taken for completion is the integration of the tablet with no palpable mass remaining in the apparatus was measured and recorded.

Result and Discussion

Characterization of drug

Description - Naringenin powder was found to be off yellow and odorless.

Solubility: Naringenin powder was found to be soluble in water, slightly soluble in ethanol and chloroform.

Taste: Naringenin powder was slightly bitter in taste.

Melting point: Melting point of Naringenin was 2510C

Phytochemical Screening

Test for carbohydrates (Benedict's test)

Orange red precipitated were observed hence it's was concluded that carbohydrate was present in the given sample.

Test for Flavonoids (alkaline reagent test)

Yellow color precipitated were observed, hence it was concluded that flavonoids were present in the given sample.

Test for Triterpenoids (Salkowski's test)

Formation of yellow color to the bottom layer not observed .and there is formation of upper layer yellow color and bottom layer white. Hence it concluded that triterpenoids were absent in the given sample.

Test for Saponins (frothing test)

There was not formation of stable foam observed hence it was concluded that saponin were absent in the given sample.

Test for tannins (ferric chloride test)

Formation of dark blue or green color not occur after adding the ferric chloride solution. hence, it's was concluded that were absence of tannin in the sample.

Test for glycosides (Keller-Kilani test)

Formation of brown ring between the layers occur hence it's was concluded that there was presence of glucosides in give sample.

Table No: 2 Flow Properties of Formulation Powder

Sr no.	Bulk density(g/cc)	TappedDensity(g/cc)	Carr'sIndex	Hausner'sRatio	Angle of Repose
F1	0.51	0.60	13.64	1.15	29.20
F2	0.50	0.62	16.09	1.19	31.25
F3	0.53	0.62	16.72	1.19	28.83

N=3 (N means no.of sample) Bulk density of the powder blends of different formulations was found to be less than 1.5. Both these values indicate good flow property and good compression characteristics.

Lamda Max (λmax)

The solutions were scanned in the range from 400-200 nm and the peaks were observed at 290 nm. The wavelength was found to be 290 nm. Which was selected for further analysis.

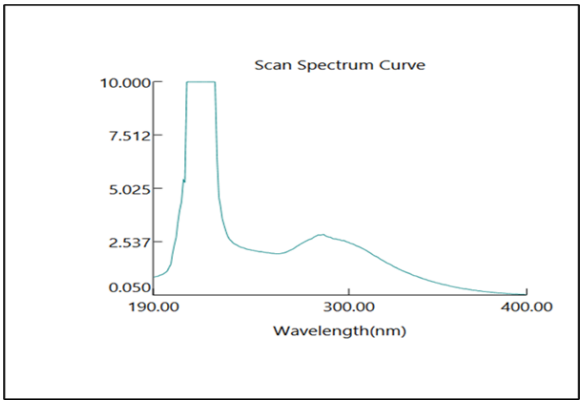

Fig No:1 Graph of Lambda Max

Tablet Thickness and Diameter

Thickness of tablet was determined by using Vernier Calliper. The thickness of the prepared tablet formulation found to be between 4.98 to5.86 mm and diameter of the tablet was found in the range of 6.98 to 7.03 mm. There was no marked variation in the thickness of the tablet within each formulation indicating uniform of powder throughout the compression process. The result of measured thickness and diameter of each tablet was shown in table

Table No: 3 Tablet Thickness and Diameter

Sr No	Tablet thickness(mm)	Tablet Diameter (mm)
F1	5.82	6.98
F2	5.86	7.03
F3	4.98	7.03

N=10 (N means no.of sample)

Weight Variation

During weight variation test none of the tablet was found to deviate by permissible percentage as per Indian Pharmacopoeia 1996 (7.5%) from the mean value of the 20 tablets. Thus, it was found that all the formulations were found to comply the weight variation test

Table No: 4 weight variation

Formulationcode	weight deviation
F1	Passes
F2	Passes
F3	Passes

N=20 (N means no. of sample)

Friability

Weight loss was calculated and represented in the term of % friability. Friability values were observed less than 1 %, indicating good strength of the tablets. Results were shown in table.

Table No: 5 Tablet Friability

Formulationcode	Friability (%)
F1	0.082
F2	0.087
F3	0.078

N=10 (N means no. of sample)

Hardness

Hardness values of the formulation ranged from 4.5 to 4.9 kg, which indicate good strength of tablet. The measured hardness of each tablet was shown in table.

Table No: 6 Hardness

Formulationcode	Hardness (kg)
F1	4.5
F2	4.7
F3	4.9

N=10 (N means no. of sample)

Disintegration time

The data for disintegration time of naringenin tablet was shown in table

Table No: 7 Disintegration time

Formulationcode	Disintegration time(sec)
F1	65
F2	76
F3	60

N=3 (N means no. of sample)

Drug release of Naringenin tablet

During in vitro dissolution study, it was found that 2 hr. time was required for the83.7% drug release from the formulation

Table No: 8 In vitro dissolutions of tablet

Time(min)	F1	F2	F3
15	26.44	21.35	27.25
30	41.62	34.16	44.58
45	55.36	68.26	72.1
60	64.11	74.07	83.7
90	76.79	78.9	81.9
120	77.09	78.10	82.4

N=3 (N means no.of sample)

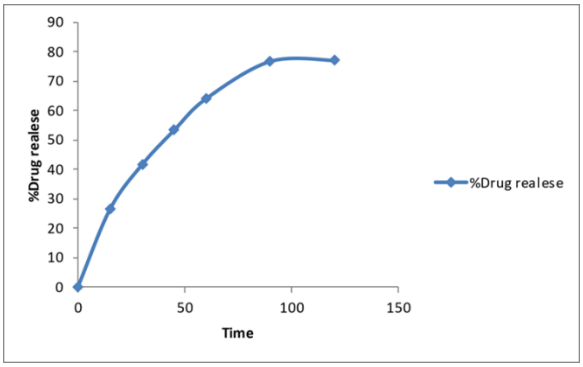

Fig No: 2 In vitro Dissolution profile of formulation F1

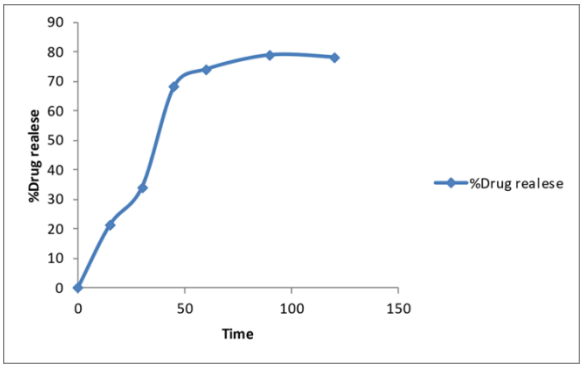

Fig No: 3 In vitro Dissolution profile of formulation F2

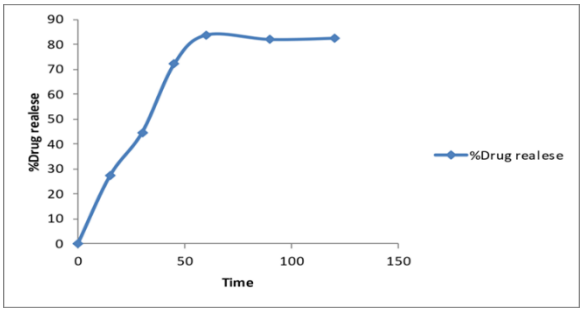

Fig No: 4 In vitro Dissolution profile of formulation F3

CONCLUSION

Free radical produces cancer ,affecting over the world population .local drug delivery via tablet formulation due to more stability and easily availability of drug from natural sources .Naringenin treat cancer by activates autophagy i.e. “self-eating” process that cells perform to allow degradation of intracellular components, including soluble proteins, aggregated proteins, organelles, macromolecular complexes and foreign bodies by inhibiting the PI3K/AKT signal, thereby inhibiting the growth of gastric cancer cells. The protective effects of naringin and naringenin result from the activation of the PI3K-Akt-mTOR pathway and the inhibition of autophagy. Therefore, flavanones cause cancer cell death by inhibiting autophagy through signaling pathways, which significantly impacts the further treatment of tumors, especially in combination with other chemical preparations. The drug Naringenin characterized for its quality by determine a λmax, which found at 290 nm. Naringenin tablet was formulated, which was qualify for the desirable quality by evaluation test. Evaluation studies performed in present investigation revealed that the formulation F3 was found to be optimum. Further dissolution profile for the formulation F3 was found to be 82.4%. Other evaluation parameters like hardness, friability was found to be 4.9 kg and 0.078% respectively for the formulation.

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