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EXTRACTION OF NARINGIN BY USING TWO DIFFERENT METHODS AND ANALYZING THROUGH UV SPECTROMETRY

***Lakpa Rinzing Sherpa**

Sikkim Skill University, Namthang, South Sikkim-737132.

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***Corresponding Author**

Lakpa Rinzing Sherpa

Sikkim Skill University, Namthang, South Sikkim-737132.

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ABSTRACT

Naringin, a flavanone glycoside abundant in citrus peels, possesses diverse pharmacological properties. This study compared solvent efficiency for extracting naringin from *Citrus reticulata* peel using maceration with methanol, ethanol, dimethylformamide (DMF), and dimethyl sulfoxide (DMSO), and Soxhlet extraction with ethanol. Two grams of powdered peel were macerated for 72 hours in each solvent, while Soxhlet extraction was carried out on 30 g peel for 4 hours. Extracts were analyzed spectrophotometrically at 276 nm against a naringin standard. DMF showed the highest absorbance (0.600), yielding approximately 2.47 mg per 2 g peel (1.24 mg/g), followed by DMSO (0.491 mg per 2 g). Methanol and ethanol gave lower yields. Soxhlet extraction produced a higher crude extract (0.9 g from 30 g; 3% w/w), but naringin isolation was not quantified. Overall, DMF proved to be the most efficient solvent for naringin extraction under these conditions.

KEYWORDS: Naringin; *Citrus reticulata*; solvent extraction; maceration; Soxhlet; UV-Vis spectrophotometry.

INTRODUCTION

Citrus peels are rich in phenolic compounds and flavonoids that offer notable health benefits. Among these, the flavanones narirutin, naringin, and naringenin exhibit strong antioxidant, anti-inflammatory, antiviral, neuroprotective, and anticancer properties. Naringenin also enhances the effectiveness of certain anticancer drugs, making it valuable for new pharmaceutical formulations. Peels additionally contain vitamin C, pectin, fiber, and essential oils, highlighting their biotechnological potential. Flavanones occur as glycosides or aglycones—naringenin and hesperetin being major aglycones—while naringin, a neohesperidoside, imparts the characteristic bitter flavor of citrus fruits.

Naringin

Naringin, a natural flavanone glycoside, was first identified in grapefruit flowers by De Vry in 1857. Its name originates from the Sanskrit word *narangi'*, meaning "orange." It occurs abundantly in citrus fruits, grapefruit, beans, cherries, cocoa, oregano, and tomatoes, reaching up to 800 mg/L in grapefruit juice. Chemically known as 4,5,7-trihydroxyflavanone-7-rhamnoglucoside, naringin exhibits diverse biological activities, including antioxidant, anti-inflammatory, antitumor, neuroprotective, and anti-ulcer effects. Although beneficial, its bitterness can limit the sensory quality of citrus-based products. Major natural sources include *Citrus aurantium*, *C. medica*, *C. sinensis*, and *Drynaria fortunei*.

Ultraviolet–Visible Spectroscopy

UV-Visible spectroscopy studies how matter interacts with electromagnetic radiation between 200–750 nm, covering both ultraviolet (UV) and visible regions. UV radiation is divided into UVA (320–380 nm), UVB (280–320 nm), and UVC (100–280 nm), while visible light spans 380–750 nm. The technique involves excitation of outer electrons, hence called electronic spectroscopy. When incident light matches the energy gap between electronic states, absorption occurs following Bohr's relation ($\Delta E = h\nu = hc/\lambda$). The absorbed energy may later be released as fluorescence, phosphorescence, or heat. Quantitatively, absorbance (A) follows Beer–Lambert's law: $A = \varepsilon bc$, where ε is molar absorptivity, b the path length, and c the concentration—forming the basis for spectrophotometric analysis in chemistry and pharmaceuticals.

Maceration Method

Maceration is a simple and low-cost conventional extraction technique that involves soaking plant material in a suitable solvent for a prolonged period. The solvent gradually penetrates the plant matrix, dissolving bioactive compounds. Factors such as solvent type, sample quantity, and extraction duration influence efficiency. Common solvents include methanol, ethanol, ethyl acetate, and water, chosen based on polarity, safety, and cost.

Soxhlet Method

Soxhlet extraction uses a continuous reflux system consisting of a flask, extractor, and condenser. The solvent repeatedly evaporates, condenses, and passes through the sample placed in a thimble, ensuring efficient compound recovery. Solvents like methanol or water are typically used, while others may be selected depending on boiling point. This method offers higher yield without requiring manual solvent replacement.

Review of Literature

1. **Isha Gupta et; al (2023)** performed QbD Based Extraction of Naringin from Citrus sinensis L. Peel and its Antioxidant Activity where they established four different extraction techniques viz. soxhlation, reflux, ultrasound-assisted extraction (UAE), and maceration were employed to extract naringin from Citrus sinensis using different solvents- ethanol, acetone, dimethyl sulphoxide (DMSO), and dimethylformamide (DMF) of varying polarity. Here they found out that concentration of naringin is more when we use UAE extraction method. They also found out that using of ethanol solvent will get the higher yield compare to methanol, DMF and DMSO.[9]
2. **Akrati Sant et; al (2022)** demonstrated extraction and hydrolysis of Naringin from Citrus fruit peels in Soxhlet apparatus where 40gms of powdered peels was taken. Methanol was employed as the solvent for the Soxhlet extraction. 55-65 ℃ temperature was set for three hours. Then, 20–25 cm3 of water were added to the methanolic extract, which had been reduced to 10–12 g under reduced pressure at 45–50 °C. This methanolic extract was stirred for 30 minutes at 60– 70 °C, then placed in a stoppered flask for 3 days with 3 cm3 of chloroform. Naringin crystals were recovered and filtered after three days.[10]
3. **Mindaugas Marksa et; al (2022)** illustrated about the Optimization of Naringin and Naringenin Extraction from Citrus × paradisi L using hydrolysis and excipients as adsorbent where raw material was allowed to warm up to 25 C. In a 250 mL RBF, a sample weighing 10.05 g was combined with the solvent in a 1:10 ratio and refluxed at 100 2 C for an hour. The mixture was centrifuged for 10 min after being allowed to cool to room temperature, and the supernatant was then decanted.[11]
4. **Juan Francisco Garcia-martin et; al (2020)** evaluated different solvents on flavonoids extraction and efficiency from sweet oranges in Soxhlet apparatus where sample was extracted with 100 ml of methanol or ethanol, respectively, and heated to 100ºC for 4 hrs. The assayed ratios of methanol and ethanol to water were 100%, 80% (80:20, v/v) and 50% (50:50, v/v), respectively. the extract was separated from solvent by using a rotary evaporator and to let the solvent evaporate.[12]
5. **Mahboubeh Tajaldinia et; al (2020)** demonstrated about the protective and anticancer effects of orange peel extract and naringin in doxorubicin treated esophageal cancer stem cell xenograft tumor mouse model in which Oranges were peeled and dried properly. Dried peels are sieved. For extraction, 20gm of citrus powder was added to 200ml of methanol and keep it for 48hrs. The mixture was filtered, and extract was evaporated to dryness by rotary evaporatorat 40 degrees Celsius.[13]
6. **Sowmya N, N Haraprasad et; al (2019)** explored about the total flavonoid content of peels of *Citrus auriantum, Citrus maxima and Citrus sinensis* using different solvents in Soxhlet Apparatus. It was set-up for 2-3 hours at a certain temperature. 15 gm of powdered drug was used for extraction by using 80% methanol. Temperature should not exceed the BP of solvent used. This is how the extraction was carried out.[14]
7. **M. David et; al in (2018)** established a simple and efficient process for the extraction of naringin from grapefruit peel waste where extraction method they used in dry albedo / hot methanol extraction in which they took 30 g of dry powder and placed in a conical flask and added 190 ml of methanol in it. After three days, filtration is done, and the methanol was extracted in a rotary evaporator at 45 degrees Celsius under decreased pressure. The extract obtained was mixed with water 20 ml and agitated at 60°C for 30 min. After adding 3 ml of dichloromethane, it was added to a flask and left at room temperature for 4 days. Filtration is done and naringin crystals were collected and dried.[15]
8. **Irina Ioannou et; al (2018)** discovered the Effect of the process, temperature, light and oxygen on naringin extraction and the evolution of its antioxidant activity in. The extraction process are as follows: Peels from fresh orange (Citrus sinensis) of Maltese variety were dehydrated using a freeze-dryer (CHRIST Alpha 1-2 LD, France) for 72 h (at 50 °C and 0.001 mbar) and then finely ground using a coffee grinder (Moulinex, France) to achieve a standard size of particles of ~ 0.315 mm. Naringin was extracted by five different methods: CSE, UAE, MAE, SCE and HPE.[16]
9. **Osama M. Ahmed et; al (2018)** Performed the Nephropreventive and Antioxidant Effects of Navel Orange Peel Hydroethanolic Extract, Naringin and Naringenin in N-Acetyl-P- aminophenol-administered Wistar Rats in which navel orange fruits were washed several times with fresh water to ensure removal of any contamination. Then, they were peeled and the peels were air dried in shade area for 20 days. The dried peels were coarsely powdered with an electrical grinder and the powder was soaked in 70% aqueous ethanol for 72 hours at room temperature. To fully mix the powder with the extraction solvent, the suspensions were allowedto be stirred frequently. The hydroethanolic extract was then filtered through Whatman filter paper and was evaporated under reduced pressure using Rotavapor to yield crude extract of navel orange peel.[17]
10. **Osama M. Ahmeda et; al (2017)** illustrated about the navel orange peel hydroethanolic extract as they dried the peels carefully by air. The dried peels were

ground to powder using a grinder. 500 g of powder were infused for 72 hours in 70% aqueous ethanol. The mixture was filtered using a Whatman No. filter paper to remove the debris particles. The residue after filtering was re-extracted under the identical circumstances to ensure complete extraction. The extract was evaporated using a rotary evaporator.[18]

11. Rui Chen, et; al (2016) explained about the therapeutic potential of naringin in which Powder was extracted by refluxing with 1 L of methanol for 2 hours, crystallization with water was carried out at 25 C with the addition of 14–15% (v/v) dichloromethane and resulted in a fivefold higher yield than conventional Hot water extraction.[19]

12. Munish Puri et; al (2011) established a Molecular characterization and enzymatic hydrolysis of naringin extracted from kinnow peel. Here higher concentration of naringin (67 mg ml−1) was obtained on boiling kinnow peel powder (5%, w/v) in water for 10 min. Further increasing boiling duration to 15 min resulted in steady state of extraction. More naringin was recovered with the increase in kinnow peel powder quantity (1–5%, w/v) for extraction. We chose 3% (KP) for carrying further experiments. Hot water was used to recover the highest yield of naringin at 90 ◦C from grapefruit peel. The method was found to be cost-effective and can easily be attempted to facilitate scale-up process. Different methods have been reported for recovery of citrus glucosides in alkaline conditions. The highest concentration of flavonoids in the citrus fruit occurs in the peel. The kinnow peel (3%, w/v) containing a concentration equivalent of 50 mg ml−1 of standard naringin (Sigma, US) was used for enzymatic hydrolysis.[20]

13. Toledo Guillen et; al (2010) performed an Extraction of Bioactive Flavonoid Compounds from Orange extraction (Citrus sinensis) Peel Using Supercritical CO2. Here orange peel was dried under hot air until 12.5% (w/w) of water content was achieved. The dried peel was ground to a particle size distribution from 1-2 mm. Extraction was carried out using a pilot scale extractor with SC-CO2 as a solvent. The optimization of the supercritical extraction conditions was determined using a Surface Response Methodology (RSM). Thus, the effects of extraction pressure, extraction temperature and cosolvent (ethanol) concentration on the yield of two flavonoids (nobiletin and tangeretin) were studied.[21]

14. Kanokorn Sudto et; al (2009) gave an efficient method for the large-scale isolation of naringin from pomelo (*Citrus grandis*) peel where 30gm powdered peel was refluxed with 200 mL of water for 4 hours, and the resulting solution was pre-concentrated using rotational evaporator under reduced pressure just to reduce volume by five times to 40 ml. The mixture was then maintained at 4 C for 36 hours and allowed to form naringin crystals. The naringin crystals were filtered. The filtrate was doubled in concentration to 20 mL and stored for 36hrs at 4 c. Crystals of naringin were collected and dried by air.[22]

15. Amelia N. Giannuzzo et; al (2003) used a Supercritical Fluid Extraction of Naringin from the Peel of Citrus paradisi where they introduced Extractions using conventional techniques where they performed in triplicate using 20 g of fragmented fresh peels. Macerations and reflux extractions were carried out with 100 mL of ethanol: water (70:30) for 30 h at room temperature (22–25°C), and for 3 h under reflux, respectively. Soxhlet extractions were performed using 200 mL ethanol refluxed for 8 h. In each case the extracts were filtered and concentrated in a rotary evaporator under reduced pressure at 40°C.[23]

16. Salmah Yusof et; al (1989) found out the content of naringin in local citrus fruits where they performed the extraction of naringin from skin. The extraction of naringin from the skin and attached membranes was done using 20g of chopped materials. The sample was macerated in a Waring Blender with methanol for 5 min. The mixture was filtered and the residue was re-extracted twice with methanol. After evaporation of the combined filtrate in vacuo, the concentrate was transferred quantitatively into a separating funnel and washed three times with a total volume of 15 ml of petroleum ether. The aqueous fraction was retained and diluted with methanol to 25 ml and the ether fraction was discarded.[24]

MATERIAL AND METHODS

Method 1: Extraction By Maceration

The orange peel was first dried and then carefully ground using a mortar and pestle to obtain a coarse powdered material. This powdered peel was further processed by sieving it through a mesh of size 40 to ensure a fine, uniform powder. Two grams of this powdered material were then weighed and individually soaked in different solvents—methanol, ethanol, DMSO, and DMF—for a period of 72 hours to allow thorough extraction of the bioactive compounds. After the soaking period, the mixtures were filtered using Whatman filter paper to separate the extract from the solid residue, resulting in clear, solvent-based extracts ready for further analysis.

Fig: 1 Dried orange peel.

Fig: 2 Sieved.

Fig: 3 Peel powder soaked in solvent.

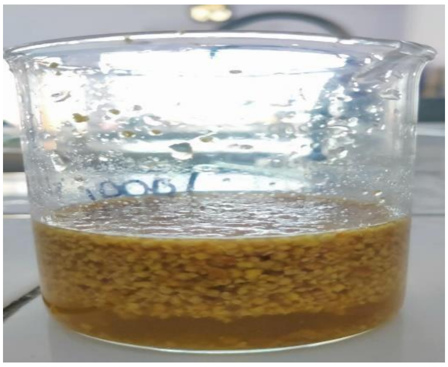

Fig: 4 Filtration soaked in solvent.

Analytical Process

The filtrate obtained from the previous step was collected for further processing, while the solid residue was discarded. A separating funnel was employed to carry out the separation process, where 10 ml of hexane was added to the filtrate to facilitate the partitioning of compounds. The yellow-coloured layer, which contained carotenoids, was discarded, and the remaining ethanolic layer was retained for subsequent analysis. To prepare the solution for measurement, a double dilution was performed. Initially, 1 ml of the separated ethanolic solution was pipetted into a 10 ml volumetric flask, and 9 ml of the respective solvent was added to make up the volume. For the second dilution, 1 ml was taken from this first dilution and transferred into another 10 ml volumetric flask, with the volume again adjusted using the same solvent. After completing the double dilution, the solution was analyzed using UV spectrophotometry to determine the absorbance, which was then used to calculate the concentration of the compounds.

Fig 5: Separation process using separating funnel.

Fig 6: UV Spectrometry.

Extraction of Standard Naringin

10 mg of the standard compound was accurately weighed and placed into a 10 ml volumetric flask. The flask was then filled up to the mark with the respective solvent, which could be methanol, ethanol, DMSO, or DMF, to prepare the standard solution. To achieve an appropriate

concentration for measurement, a dilution was performed by transferring 1 ml of this solution into another 10 ml volumetric flask, and the volume was adjusted with the same solvent, completing a double dilution process. After this, the absorbance and corresponding wavelength of the solution were measured using a UV spectrophotometer, and the concentration of the standard was subsequently calculated based on these readings.

Method 2: Soxhlet Extraction

30 gm of powdered orange peel was taken and subjected to reflux with 100 ml of ethanol for four hours at 80°C. The resulting solution was then concentrated using a rotary evaporator under vacuum to remove the solvent partially. The concentrated extract was transferred into a China dish and stored appropriately for further analysis.

Fig 7: Soxhlet Extraction.

RESULT AND DISCUSSION

Method 1: UV-Visible Spectrophotometric Analysis

The absorbance values of the orange peel extracts and standard naringin in different solvents were recorded using UV-Vis spectrophotometry. The results are summarized in Table 1.

- In ethanol, the sample extract showed an absorbance of 0.141 at 264 nm, whereas the standard naringin exhibited an absorbance of 0.223 at 276 nm
- For DMF, the sample extract had a higher absorbance of 0.600 at 276 nm compared to the standard's 0.243 at 276 nm.
- Methanol extract showed an absorbance of 0.159 at 264 nm, while the standard had 0.276 at 275 nm.
- In DMSO, the sample extract exhibited an absorbance of 0.160 at 277 nm, whereas the standard recorded 0.326 at 276 nm.

Table 1: Absorbance of sample extract and standard naringin in different solvents.

ABSORBANCE	ETHANOL	DMF	METHANOL	DMSO
Sample Extract	0.141	0.600	0.159	0.160
Standard naringin	0.223	0.243	0.276	0.326

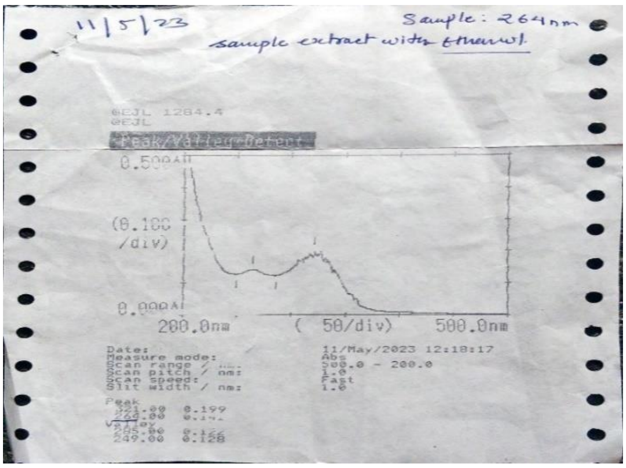

Fig 8: Ethanol (sample)

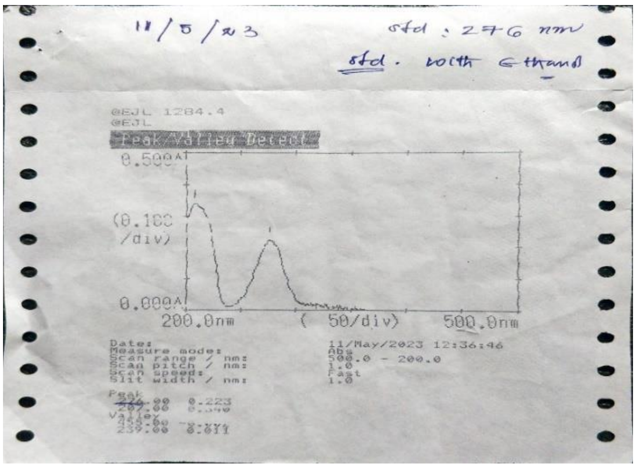

Fig 9: Ethanol (Standard)

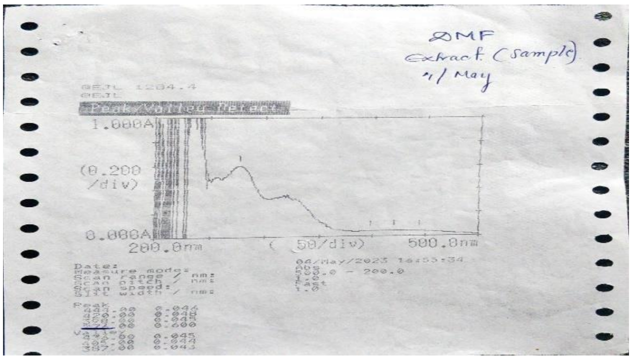

Fig 10: DMF (Sample)

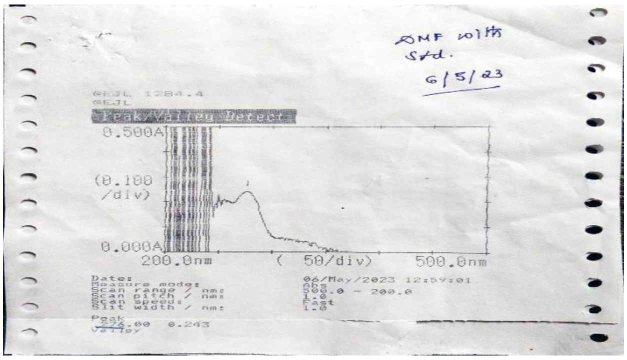

Fig 11: DMF (Standard)

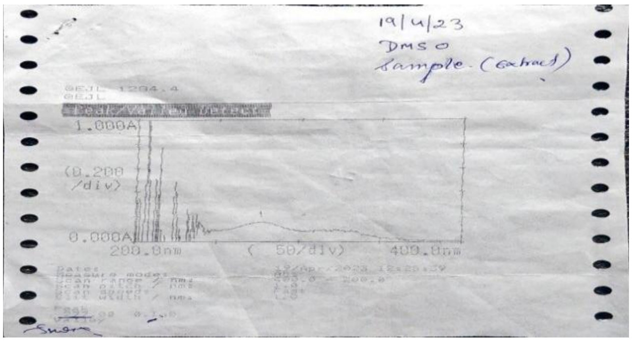

Fig 12: DMSO (Sample)

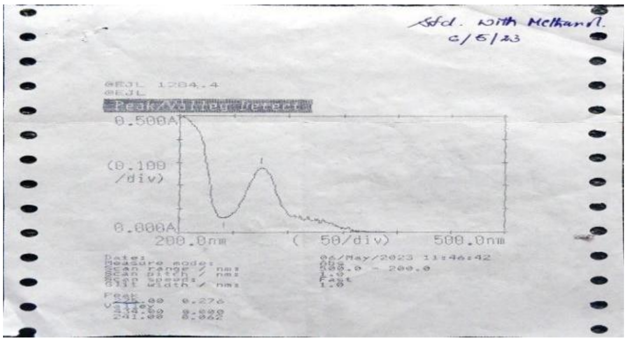

Fig 13: Methanol (Standard)

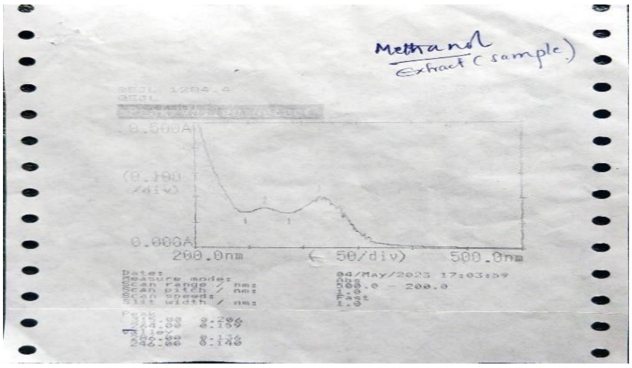

Fig 14: Methanol (Sample)

The concentration of naringin in the extracts was calculated based on the absorbance of the standard. In DMF, 2 g of powdered peel contained 2.469 mg of naringin, while in DMSO, the same amount of peel contained 0.4907 mg of naringin. These results indicate that DMF was the most efficient solvent for extracting naringin under the conditions tested.

Method 2: Solvent Extraction and Yield Determination

The orange peel extract obtained via reflux in ethanol was concentrated and weighed. The weight of the extract was found to be 0.9 g from 30 g of powdered peel, corresponding to a percentage yield of 3%. This data is presented in Table 2.

Table 2: Extract yield of naringin from powdered orange peel.

Sample	Weight of Extract (g)	Percentage Yield (%)
Orange Peel (30g)	0.9	3

These results demonstrate that ethanol reflux extraction yields a small but quantifiable amount of naringin. Method 1 (UV-Vis analysis) provides more specific information on naringin content in different solvents, while Method 2 gives a direct measure of total extract yield.

DISCUSSION

Naringin was estimated by two different extraction methods and was further analyzed through UV spectroscopy to determine the absorbance and concentration. Two extraction technique includes maceration and Soxhlet. Maceration was done by using four different solvents which includes Ethanol, Methanol, DMF and DMSO. The absorbance was determined as 0.141, 0.159, 0.600, 0.160 at 264nm, 264nm, 276 nm, 277nm respectively during sample extraction. For standard Naringin the absorbance was determined as 0.223, 0.276, 0.243, 0.326 at 276nm, 275 nm, 276nm, 278 nm. From the above-mentioned wavelength and absorbance, DMF and DMSO showed the peak near to Standard naringin. Therefore, the concentration for DMF was found to be 2.4 mg and for DMSO was0.49 mg. So, it is evidently found that DMF is giving better yield than DMSO. Another method was done through Soxhlet extraction using Ethanol as a solvent. 30 gm of powdered peel was taken from which extract determined was 0.9 gm.

Further, isolation method would give us an appropriate value of naringin.

CONCLUSION

Naringin, a bioflavonoid is present in peels of citrus fruit possesses many pharmacological properties. Naringin till date has shown to be potent anti- inflammatory, anti-tumor, antiviral, antibacterial, antioxidant and cardio protective agents and it has also shown to increase the bioavailability of calcium channel blocker.

Two different extraction methods: **Maceration** and **Soxhlet extraction** were performed using different solvent systems like Ethanol, Methanol, DMF and DMSO. In the maceration process DMF was found to give a higher yield of about 2.46mg than compared to the other solvent system. Similarly, for the Soxhlet extraction process Ethanol was used as a solvent system. As comparing the different solvents system and its availability, DMF can be further used for the estimation of naringin from the peels of the *Citrus reticulata Blanco* Furthermore, the physical parameters of the extract are yet to be determined.

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