RESEARCH PAPER

Evaluation of Antioxidant Potential of Solvent Extracts of Frutis of Fortunella Japonicum

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ABSTRACT

The present work aimed at exploring the antioxidant potential of Fortunella japonicum fruit The present study investigated the antioxidant potential of solvent extracts of Fortunella japonicum fruits. Successive extraction using petroleum ether, chloroform, ethanol, and water yielded resinous extracts with varying phytochemical profiles. Preliminary screening confirmed the presence of alkaloids, phenolics, flavonoids, tannins, proteins, and carbohydrates depending on the solvent system. Quantitative analysis revealed significant levels of phenolics (up to 266.53 GAE mg/g in ethanol extract) and flavonoids (483.05 QE mg/g in petroleum ether extract). HPTLC analysis indicated the presence of major phytoconstituents such as α/β-pinene, limonene, and linalool. Antioxidant activity assessed by DPPH radical scavenging assay demonstrated that the ethanol extract exhibited the lowest IC50 value (844.31 µg/mL), suggesting strong free radical inhibition. These findings highlight Fortunella japonicum fruits as a promising source of natural antioxidants, supporting their potential role in mitigating oxidative stress-related disorders.

Keywords: Analgesic, Anti-Oxidant, Fortunella Japonicum, Phosphomolybdenum, Dpph

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Introduction

Every civilization in the world has utilized medicinal plants as a possible source of pharmaceuticals and therapeutic agents.[1] Around 80% of people in Asia, Africa, and Latin America are thought to use traditional medicine, according to the World Health Organization (WHO)[2]. Antioxidants are chemicals that prevent or slow down free radical reactions and prevent or delay cellular damage[3]. They are in charge of the body's defence mechanisms against diseases linked to free radical attack. Therefore, consuming antioxidants produced from plants helps prevent degenerative diseases like Parkinson's, cancer, Alzheimer's, and atherosclerosis[4] that are brought on by oxidative stress. Within a biopsychosocial paradigm, the human body's defence mechanisms for preserving its integrity and shielding itself from harm include the ability to feel pain[5].

Fortunella japonica, a member of the Rutaceae family, is abundant in limonene, ß-sitosterol, umbelliferone, epigenin, and other compounds[6]. The research made it clear that traditional practitioners have utilized a number of herbs and their components for their anti-oxidant properties and ability to effectively treat pain. A common plant in Asia, Fortunella japonica has recently been studied for a number of pharmacological properties, including as antibacterial, anti-inflammatory, antioxidant, and hypotensive properties[7–11]. We investigated the antioxidant impact of the different solvent extracts of Fortunella japonica fruits since the plant extract included flavonoids and phenolics.

Material and Methods

Collection of plant material

The plant of *Fortunella japonicum* were collected from the Lake View Nursery, Madhya Pradesh in the month of December and authenticated at MFP-PARC, Bhopal.

Extraction of phytoconstituents[12]

The powdered fruits were used for the extraction process by hot continuous extraction method using soxhlet apparatus. 169.35 g of fruit powder was evenly placed in the extractor of the apparatus and 340 mL of methanol was poured over it and was allowed to

collect in the attached flask. Extraction was achieved by heating the solvent at 65°C for 9 h till a colorless solution was collected in the siphon tube of the apparatus. The successive solvent extraction of the defatted material was done using chloroform, ethanol and purified water. The aqueous extraction was achieved by cold maceration method. All the extract containing solvents were filtered through Whatman filter and concentrated using rotary vacuum evaporator. The resinous extracts were collected and stored in desiccator to remove the excessive moisture**.** The dried extracts were stored in desiccator for further processing.

Preliminary phytochemical screening[13]

The extracts were evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in it. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests.

Total Phenolic Content[14]

For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After 3 min, 300 µL of 20% aqueous Na_2CO_3 solution was added to it and the mixture was allowed to settle for 2 h. The absorbance was measured at 760 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (20-100 ppm) were treated similarly to obtain the calibration curve. The control solution contained 200 µL of water and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Total Flavonoid Content[14]

Determination of total flavonoids content was based on aluminium chloride method. 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25- 150µg/ml were prepared in methanol. 0.1 g of dried extract was extracted with 10 ml methanol, filtered, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm.

High Performance Thin Layer Chromatography (HPTLC)

In order to achieve separation of the standards on the percolated HPTLC plates, different solvents are used such as ethyl acetate, n-butanol, toluene, and methanol in the different ratios were used, but the separation was not achieved. The separation occurs using chloroform and hexane in the ratio of (8:2 v/v).

The chromatographic conditions included 10 mL of mobile phase chloroform and hexane in the ratio of (8:2 v/v), Loba chem TLC Plate Silica Gel 60 F 254 10*10 cm stationary phase, Anisaldehyde as the derivatizing agent, 366 and 254 nm for visualization of plates, temperature and saturation time of 25°C and 30 min respectively and syringe volume of 2µL.

***In vitro* antioxidant activity of extract by DPPH assay**[15]

The antioxidant action of the extracts of *Fortunella japonicum* was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The free radical scavenging activity of the synthesized molecules was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The test samples (100 µL, 100-500 µg/mL) were prepared in DMSO and were mixed with 1.0 mL of DPPH solution and filled up with methanol to a final volume of 4 mL. Absorbance of the resulting solution was measured at 517 nm in a visible spectrophotometer. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated.

Results and Discussion

Extraction and phytochemical screening

The powdered plant material were accurately weighed and filled in a cellulose thimble and successively extracted with solvents of varying polarity. The dry extract was obtained by evaporating the solvent and the yield was calculated (Figure 1).

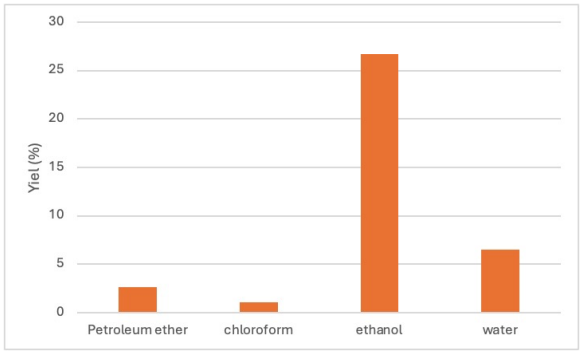

Figure 1. Percent yield of extract

Table 2 Phytochemical screening of *F. japonicum* extract

Chemical Tests	**Pet. Ether extract**	**Chloroform extract**	**Ethanolic extract**	**Aqueous extract**
Mayer's reagent	-	+	+	-
Hager's reagent	-	+	+	+
Wagner's reagent	-	+	+	+
Dragendorff's reagent	-	+	+	+
Froth test	+	-	-	-
Kedde's Test	-	-	-	-
Bontrager's Test	-	-	-	-
Keller-Kiliani	-	-	-	-
Ferric chloride	-	-	+	+
Gelatin Solution	-	-	-	+
Alkaline reagent test	+	-	+	+
Vanillin HCl test	+	+	+	-
Shinoda test	-	-	+	+
Alkaline reagent test	+	-	-	-
Zinc HCl reductino test	-	+	+	+
Millon's Test	-	-	-	+
Ninhydrin Test	-	-	-	+
Liberman-Burchard Test	-	+	+	-
Salkowski Test	-	+	+	+
Molischs Test	-	-	-	+
Barfoed Test	-	-	-	+

The observations suggested the presence of flavonoids, phenolics and sterols in the all the whereas the petroleum extract revealed positive test for saponins. The aqueous extract was found to contain protein and carbohydrates in it. Alkaloids were found to be present in all the extracts except petroleum ether extract.

Total phenolic and flavonoid content

The extracts of *Fortunella japonicum* leaves were evaluated for quantifying the total phenolic content. Standard curve of gallic acid was plotted in distilled water. The result of the total phenolic content of the extract examined using Folin-Ciocalteu method (Table 2). Standard curve of quercetin was plotted for determination of total flavonoid content. The result of the total flavonoid content of the extract examined using Aluminum chloride method (Table 2).

Table 2. Total Phenolic and Flavonoid Determination

Extract	Total Phenolic Content		Total Flavonoid Content	
	Absorbance at 760 nm	GAE mg/g	Absorbance 420 nm	QE mg/g
Petroleum Ether	0.152	30.41	2.853	483.05
Chloroform	0.626	127.14	2.748	465.25
Ethanol	1.309	266.53	2.166	366.61
Water	0.256	51.63	1.66	280.85

HPTLC of the extracts

The HPTLC imaging of the extracts in white light as well as 254 nm and 366 nm was recorded. The HPTLC chromatogram revealed the presence of three major components at Rf 0.12 (probably α/ß-pinene), 0.17 (probably limonene) and 0.82 (probably linalool) in the extracts (Figure 2).

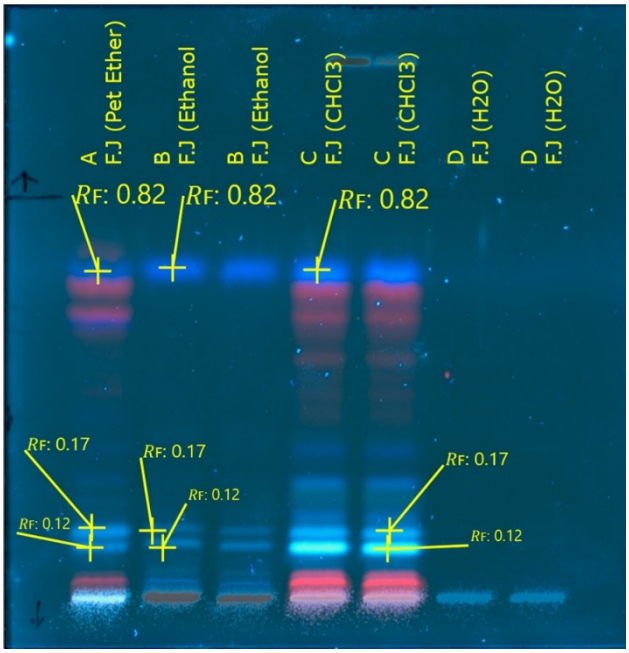

Figure 2. HPTLC chromatogram of extracts

Antioxidant activity by DPPH radical inhibition

The absorbance of control (DPPH + methanol) as well as various concentration of the test solution was measured at 517 nm using UV-visible spectrophotometer and the % DPPH inhibition was measured (Table 3).

Table 3. Percent DPPH Inhibition

	Petroleum Ether	Chloroform	Ethanol	Water
100 µg/mL	0.64	11.17	2.33	0.94
200 µg/mL	1.78	13.27	11.55	4.60
300 µg/mL	2.68	14.98	19.66	10.85
400 µg/mL	4.20	17.48	20.15	16.27
500 µg/mL	7.52	23.39	29.61	24.88

The IC_{50} value was calculated and it was found that the lowest IC50 was obtained for the ethanol extract. The IC50 was 3324.47 µg/mL, 1068.83 µg/mL, 844.31 µg/mL and 1004.82 µg/mL for the petroleum ether, chloroform, ethanol and aqueous extracts respectively.

Conclusion

The study confirmed that *Fortunella japonicum* fruit extracts possess notable antioxidant activity, attributable to their rich phenolic and flavonoid content. Among the tested solvents, ethanol extract demonstrated the most potent radical scavenging effect, while petroleum ether extract showed the highest flavonoid concentration. The phytochemical diversity revealed through screening and HPTLC analysis underscores the therapeutic potential of this plant. Given its antioxidant efficacy, *Fortunella japonicum* may serve as a valuable candidate for developing natural remedies against oxidative stress-induced degenerative diseases. Further in vivo studies and mechanistic investigations are warranted to validate its pharmacological applications and explore its role in pain modulation and other biological activities.

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