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Antifungal and Cytotoxic Activities of *Hibiscus rosa-sinensis* Flower Extract: A Pharmacological Study

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Abstract: This study aimed to examine the pharmacological properties of *Hibiscus rosa-sinensis* flower extract, including its cytotoxic effect on MCF-7 breast cancer cells. Initially free radical scavenging activity was done to identify the potential extract of the study and antioxidant activity was determined. The DPPH assay showed that aqueous flower extract of *H. rosa-sinensis* exhibited maximum radical scavenging activity. Total antioxidant activity by phosphomolybdenum assay and lipid peroxidation by thiobarbituric acid reactive substance study exhibited the strong antioxidant capacity of the extract. Qualitative analysis identified flavonoids, phenols, alkaloids, terpenoids, and tannins, with significant phenolic (0.713 mg of GAE) and flavonoid content (0.117 mg of Quercetin) for 1 mg of the extract. Antifungal activity showed inhibitory effects against *Rhizopus* and *Penicillium* in a dose-dependent manner. The extract also demonstrated potent cytotoxicity against MCF-7 breast cancer cells, highlighting its potential in cancer treatment. These findings unveiled the pharmacological evaluation of *Hibiscus rosa-sinensis* flower extract as a valuable source of therapeutic approaches.

Keywords: *Hibiscus*, Antioxidant, MCF-7, DPPH assay, Quercetin, Antifungal, Cytotoxic

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Introduction

Natural products have consistently served as the foundation of healthcare and the Global estimates indicate that no less than 80% of individuals worldwide rely on natural products for various aspects of primary healthcare (Msomi and Simelane, 2019). The perception of natural medicines as safer alternatives to synthetic drugs has led to a remarkable increase in human exposure to a wide range of natural products, including plants, phytotherapeutic agents, and phytopharmaceutical products. Consequently, there has been a resurgence of scientific interest in exploring the biological effects of these natural products. The structural diversity of natural products plays a pivotal role in the development of numerous modern drugs (Ekor, 2014). Medicinal

plants harbour a diverse array of secondary metabolites including organic acids, aromatic compounds, terpenoids, steroids, flavonoids, alkaloids, carbonyls, and more, that exhibit a broad range of pharmacological activities. Despite secondary metabolism typically constituting less than 10% of the overall plant metabolism, its products comprise the primary constituents of plants with pharmaceutical properties (Pagare *et al.*, 2015). The phytochemicals found in plants effectively act as medicinal agents, making plants an enduring source of medicine for various diseases and disorders, that has been utilized for thousands of years. The breakthroughs achieved by studying plant extracts highlight the importance of harnessing the power of nature's pharmacy and leveraging the diverse chemical compositions found in natural products with therapeutic potential (Rabizadeh *et al.*, 2022).

Excessive generation of free oxygen and nitrogen species or their inefficient quenching in the cell leads to oxidative stress, which serves as a major driving factor in the initiation and progression of various disorders, including cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases. In a state of normal physiology, the body's intricate antioxidant defense system, involving enzymatic and nonenzymatic pathways, establishes a stable balance between prooxidants and antioxidants, which is essential for maintaining overall well-being (Sharifi-Rad *et al.*, 2020). Antioxidants possess the capability to mitigate cellular damage caused by free radicals and reactive oxygen species. Evaluating antioxidant activity as a research approach is crucial for establishing the scientific foundation behind traditional herbal medicines that are renowned for their purported antioxidant properties. The growing interest in natural antioxidants stems from their favourable safety profile in comparison to synthetic agents (Hong *et al.*, 2021).

Invasive fungal infections, whether localized or systemic, are predominantly caused by *Candida albicans*, presenting a significant and multifaceted threat to public health in terms of both medical and economic implications. These infections are characterized by high mortality rates, increased healthcare costs, and prolonged hospital stays, underscoring the urgent need for effective prevention and treatment strategies. A variety of antifungal agents, including polyenes, pyrimidines, echinocandins, and triazoles, have been developed and are currently used for treating invasive fungal infections. Among them, fluconazole and voriconazole are widely prescribed antifungals. However, a persistent challenge in the field is the ongoing development of resistance by pathogenic microorganisms towards these drugs (Alyousef, 2021). In recent years, the search for alternative antifungal drugs has intensified due to the undesirable side effects, toxicity, drug-drug interactions, resistance development, and declining effectiveness associated with many existing antifungal medications. The pharmaceutical industry recognizes the significance of natural products in drug development programs (Hossain *et al.*, 2022). Consequently, extensive studies have been conducted on numerous medicinal plants, aiming to discover safer, less toxic, and more potent antifungal drugs. Numerous medicinal plants have been identified as valuable reservoirs of natural antimicrobial compounds, offering a potential alternative for the effective treatment of challenging bacterial infections. The World Health Organization acknowledges medicinal plants as the optimal source for a diverse range of drugs (WHO, 2022).

For more than five decades, the pivotal role of natural compounds in governing cellular proliferation and differentiation has been recognized, although their significance as regulators of cell death has gained appreciation only in recent years. The acknowledgment of natural products as influential modulators of the crucial pathways implicated in cancer development and progression has revitalized interest in their potential as chemopreventive and chemotherapeutic agents against cancer (Deniz *et*

al., 2017). Despite the numerous treatments already derived from natural sources, there is still an abundance of untapped potential within nature's vast repertoire.

The traditional use of *Hibiscus* flower has long been recognized for its pharmacological potential in treating disorders like hypertension (Farazi and Tarkhani, 1999), and reported to possess antioxidant, hypolipidemic (Ochani and D'Mello, 2009), anti-tumor properties (Tseng *et al.*, 2000; Lin *et al.*, 2007; Arullappan *et al.*, 2013; Chiu *et al.*, 2015), antidiarrhetic, antiphologistic (Shimizu *et al.*,1993), antispermatogenic and androgenic (Jana *et al.*, 2013), anticonvulsant activities (Reddy *et al.*, 1997). A decoction of the flowers is employed for hemoptysis, catarrhal bronchitis, and dysmenorrhea. They are also applied externally to treat sores, chronic ulcers, scabies, and certain types of dermatitis. Additionally, the fresh juice of the flower has been found to provide protection against electro-convulsions (Kholkute and Udupa, 1976; Saha *et al.*, 2008). These findings provide further support for the idea that a comprehensive hibiscus extract could potentially contain well-tolerated compounds with pharmacological properties. The plant kingdom comprises numerous species that produce diverse bioactive compounds with varying chemical structures. The species diversity of plants contributes to variations in their medicinal properties, which can vary based on the region and time of collection. Different plant species may contain unique chemical compositions and bioactive compounds, leading to variations in their therapeutic effects. Additionally, environmental factors and geographic location, can influence the growth and development of plants, thereby affecting the accumulation of active constituents responsible for their medicinal properties (Sharma *et al.*, 2020). Therefore, the present study aimed to assess the phytochemical, antioxidant, antifungal, and cytotoxic effects of *H. rosa-sinensis* flower extract to confirm its pharmacological activity, regardless of species diversity and geographical location.

Materials and Methods

Sample preparation:

The flowers of *Hibiscus rosa-sinensis* were collected from Vellore district, Tamil Nadu, washed and air dried at room temperature for 10 days. They were then ground into a coarse powder and stored in airtight containers for future use. The preparation of the aqueous extract involved soaking the dried flower sample in an equal volume of solvent and allowing it to stand at room temperature for 48 h. Afterward, the extracts were filtered, made into a crude powder, and utilized for the analysis.

Antooxidant assay:

Free radical scavenging activity using DPPH:

Free radical scavenging activity assay was performed using DPPH as described by Brand-Williams *et al.* (1995). 1 ml of 0.1 mM DPPH solution in methanol was mixed with 1 ml of various concentrations of flower extract (100 µg) using various solvents. Mixture of 1 ml methanol and 1 ml DPPH solutions were used as control. The decrease in absorbance was measured at 517 nm after 30 min in dark using UV-Vis spectrophotometer. Ascorbic acid was used as reference standard and the % inhibition was calculated.

Total antioxidant activity by phosphomolybdenum method:

The assay was performed as described by Prieto *et al.* (1999). One milliliter of each sample extract of varying concentration range (10-50 mg/ml) was mixed with 3 ml reagent solution (0.6 M H_2SO_4, 28 mM Sodium phosphate and 4 mM Ammonium molybdate). The blank solution contained 4 ml reagent solution only. The mixtures were incubated at 95 °C for 150 min. After the mixture had cooled to room temperature, absorbance was measured at 695 nm and the antioxidant capacity was calculated based on standard equation.

Measurement of Lipid peroxidation by thiobarbituric acid reactive substance (TBARS)

assay:

Lipid peroxidation inhibition assay was performed by the procedure given by Ke *et al.* (1984) . In brief, 10 µl of sample at varying concentrations (10-50 µg/ml) and 40 µl of 20 mM phosphate buffer (pH 7.0) were added to an eppendorf tube on ice. In each tube, 50 µl of 3% sodium dodecyl sulfate (SDS), 200 µl of 0.1 N HCl, 30 µl of 10% phosphotungstic acid, and 100 µl of 0.7% of 2-thiobarbituric acid (TBA) were added. The tubes were firmly closed and boiled at 100°C for 30 min in water bath. The reaction mixture was mixed with 400 µl of n-butanol and then centrifuged at 3000 rpm for 10 min. Supernatants were collected and loaded in a 96-well plate and read at 515 nm/555 nm using microplate reader.

Qualitative Phytochemical analysis:

The crude extract of plant sample (10 mg/ml) was subjected to phytochemical examination, such as flavonoid, alkaloid, tannin, phenol, terpenoid, saponin, glycosides and steroids using standard reagents.

Quantitative Phytochemical analysis:

Total Phenolic Content Determination:

The total phenolic content of the extract (Lister and Wilson, 2001) was determined using Folin-Ciocalteu reagent. The calibration curve's reference standard for plotting was gallic acid. To 0.5 ml of diluted (1:10) Folin-Ciocalteu reagent, 0.1 ml of the crude extract (10 mg/ml) was added. The mixture was then neutralised with 0.4 ml of 2% sodium carbonate solution and then incubated at 45°C for 30 min. Using a double beam UV-VIS spectrophotometer, the absorbance of the resultant blue colour was determined at 650 nm. From a standard curve made with gallic acid and its linear equation, the total phenolic contents were calculated. The amount of total phenolic components in dry extract is given as mg/g gallic acid equivalent (GAE).

Total flavonoid content (*TFC*) *determination*:

Aluminium chloride method was used to examine the total flavonoid content of a specific plant extract (Zhishen *et al.*, 1999). Methanol was used to dilute the extract sample. In order to create the calibration curve, quercetin was diluted in methanol. 0.2 ml of Quercetin and the diluted extract (10 mg/ml) were combined with 0.04 ml of potassium acetate solution and 0.04 ml of 10% aluminium chloride solution. For 30 min, the mixture was left at room temperature. Then, a UV-VIS spectrophotometer was used to detect the mixture's maximum absorbance at 420 nm. Total flavonoid content was calculated as milligram of quercetin equivalent per gram.

Determination of Antifungal activity by well diffusion method:

Agar well diffusion (NCCLS, 1993) was used to test the antifungal activity of given sample. A sterile swab moistened with the fungal suspension was used to spread on czapek dox plates. Following that, 8 mm diameter wells were punched into the agar medium, loaded with varying concentrations of aqueous flower extract (1000 µg, 500 µg, 250 µg and 100 µg) and allowed to diffuse at room temperature for 2 h. The plates were then incubated upright at 25-28 °C for 48-72 h. Fluconazole (30 µg/ml) was used as standard antifungal agent. The diameters of the growth inhibition zones were measured in millimetres after incubation. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. The plates were then incubated at 25-28°C for 48-72 h. The antifungal activity was assayed by measuring the diameter of the zone of inhibition in mm formed around the well.

Cell Culture:

The breast cancer cell line MCF-7 was purchased from NCCS, Pune and were cultured in low glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and antimycotic solution. The cells were maintained under standard culture conditions in

an incubator at 37 °C with 5% CO_2 and 95% humidity.

Morphology of MCF-7 cells and cell viability by MTT:

A phase-contrast microscope (Nikon Eclipse TE 2000; Nikon Corporation, Tokyo, Japan) was used to observe the morphology of MCF-7 cells exposed to *H. rosa-sinensis* extract. The cytotoxicity of *H. rosa-sinensis* extract on MCF-7 cells were determined using the MTT dye uptake technique (Igashi and Miyazawa, 2001). MCF-7 cells were seeded at a density of 1×10^5 cells/well in a 96-well plate and incubated for 24 h at 37°C. Subsequently, MTT solution (5 mg/ml) was added to each well and incubated. After reaching 80% confluency, the cells were treated using varied dose-ranges of the extract (10 µg/ml to 110 µg/ml) at 24 h time interval. After incubation, the cells were washed and treated with 200 µl of isopropanol in dark and the OD value was measured using a Bio-Rad microplate reader (Model 550) at 570 nm. The percentage viability was calculated by comparing the OD values to the control, and the sample's maximal inhibitory concentration (IC_{50}) was determined from a standard graph.

Statistical analysis:

All the tests were done in triplicates(n=3) and were expressed as mean ± standard deviation. Statistical analysis was done by one-way ANOVA. In all cases statistical significance was set at $p < 0.05$.

Results and Discussion

Medicinal plants are extensively employed in traditional medicine globally (Atanasov *et al.*, 2015). Their diverse applications have also piqued the interest of modern medicine. These plants serve as a valuable source of biologically active compounds that hold potential for the prevention, delay, or treatment of various diseases and disorders. Despite recent developments in synthetic chemistry, bioactive plants or their extracts continue to contribute significantly to the discovery and production of new and novel drugs for treating and preventing disease. As a source of new and novel chemotherapeutics, plants have almost unlimited potential that hold pharmaceutically active compounds (Thomford *et al.*, 2018). Exploring plants, particularly those traditionally consumed as nutrients or food additives, presents an untapped opportunity for discovering new medicinal substances. Hence, in the context of seeking alternative medicine, particularly in search of cytotoxic and antioxidant agents, the present study aimed to demonstrate the pharmacological properties of *H. rosa-sinensis* flower extract.

Radical scavenging activities are very important due to the deleterious role of free radicals in foods and in biological systems. Antioxidants are vital for immune and inflammatory responses, serving as redox signaling mechanisms that sense even minimal oxidative stress. They activate protective defence system and maintains the protein integrity. Recently, there has been notable interest in exploring plant-derived constituents for antioxidant mechanism (Ponnampalam *et al.*, 2022). Diverse *in vitro* methods exist for evaluating antioxidant activity and radical scavenging ability. Antioxidant capacity serves as a valuable parameter for assessing medicinal bioactive components. Various synthetic and biological free radical species like DPPH radical, are commonly employed in these assessments (Mandale *et al.*, 2011). Natural occurring antioxidants have been increasingly sought for use in medicinal materials to replace synthetic antioxidants restricting their use because of their potentially carcinogenic side effects (Afify and Hassan, 2016).

DPPH radical scavenging activity:

The DPPH assay measures drug reactivity in the presence of a stable free radical. When DPPH combines with hydrogen-donating molecules, absorption decreases, resulting in the formation of the reduced form (Diphenyl picryl hydrazine: non-radical) and the decolorization of the violet colour. DPPH method is effective to screen excess amount of samples and is independent of solvents polarity

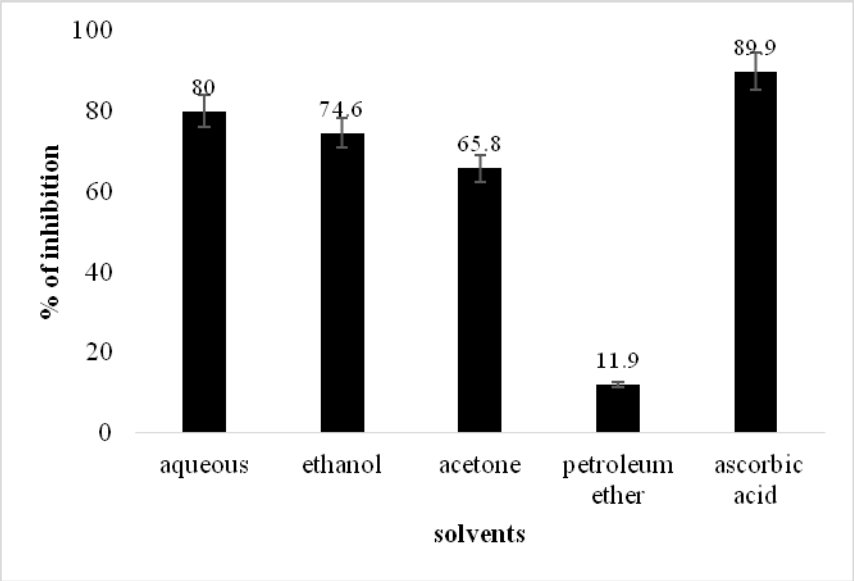


Fig. 1: DPPH free radical scavenging activity of flower extract in different solvents.

(Marxen *et al.*, 2007). Scavenging activity of different extracts of *H. rosa-sinensis* against DPPH radical at varying concentration (100 µg/ml) was determined and the results are shown in Figure 1. The aqueous extract of *H. rosa-sinensis* exhibited maximal scavenging activity with 80% of radical inhibition at a concentration of 100 µg/ml, while ethanol extract revealed 74.6% of inhibition. The strength of the scavenging ability of flower extracts of *H. rosa-sinensis* was observed to be water>ethanol>acetone>petroleum ether. There will be a certain amount of colour change based on the quantity and strength of the antioxidants. Significant free radical scavenging activity of the extracts is shown by a considerable drop in the reaction mixture's absorbance (Lalhminghlui and Jagetia, 2018). The standard ascorbic acid showed strong radical scavenging activity. Basically, it is necessary to identify a solvent that makes the extracts more soluble. Compounds dissolve more readily in solutions with a higher solubility. Samples can be validated for their therapeutic effects only when they interact with cells across a medium (Swathi *et al.*, 2022). The highest radical quenching ability of aqueous extract of *H. rosa-sinensis* might be due its absolute soluble nature and hence, aqueous extract was used for further studies.

Plant extracts have been frequently assessed for their total antioxidant capability using the phosphomolybdate technique, where Mo (VI) is converted to Mo (V) in the presence of extracts and forms the green colour phosphomolybdenum V complex (Prasad *et al.*,2009). The total antioxidant activity of aqueous extract of *H. rosa-sinensis* at varying concentrations revealed that, the extract possessed its maximum total antioxidant potential at its highest concentration of 50 mg/ml as that of standard ascorbic acid (Fig. 2). This might be because the extract could have significant levels of total phenolics and flavonoids. Phenolic compounds' redox characteristics make them useful for quenching singlet and triplet oxygen, neutralising free radicals, and breaking down peroxides (Osawa, 1994).

To further confirm the antioxidant activity of *H. rosa-sinensis*, the extract was tested for its ability to prevent ROS-mediated damage to lipids and the results are presented in Figure 3. The biomolecules that are more vulnerable to oxidative stress include lipids, and the byproducts of lipid peroxidation are thought to be possible

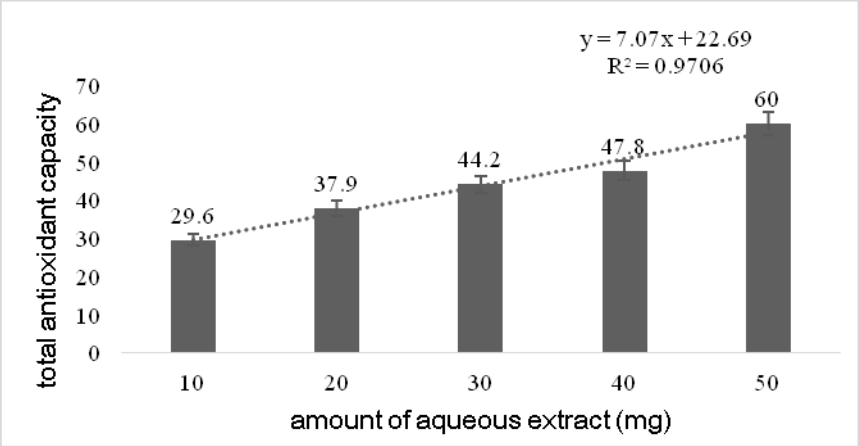


Fig. 2: Total antioxidant activity of aqueous extract

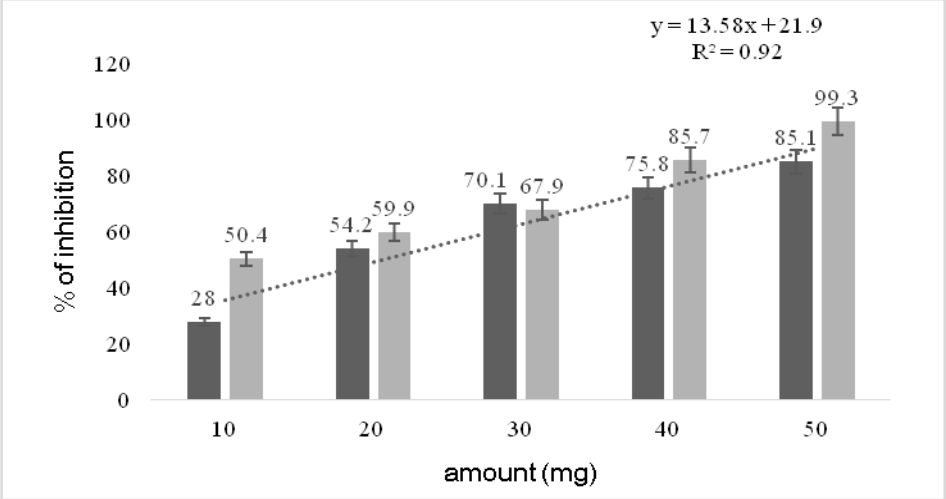


Fig. 3: Measurement of lipid peroxidation inhibition of aqueous extract.

biomarkers for oxidative stress and related illnesses (Radha and Sumathi, 2022). Cells may be damaged by lipid peroxidation caused by oxygen radicals, which generates a cytotoxic and mutagenic malondialdehyde (MDA) and conjugated diene compounds. Lipid peroxidation spreads rapidly, damaging lipidic molecules due to its radical chain reaction nature (Pizzino *et al.*, 2017). The life time of lipid peroxidation is extremely short (10^{-9} s at 30 °C) (Pizzino *et al.*, 2017). Lipid peroxidation (LPO) is an indicator of tissue degeneration, thereby losing its functional ability. Hence, it is necessary to study the protective effects of *H. rosa-sinensis* against oxidative stress-stimulated deterioration to biomolecule. Incubation of aqueous flower extract with oxidizing system exhibited that, *H. rosa-sinensis* were able to dose-dependently inhibit the oxidative damage of lipid up to 85% at its maximum concentration (50 mg/ml). Lipid peroxides are unstable and can break down into a wide range of substances, including reactive carbonyl compounds. Aqueous extract of *H. rosa-sinensis* improved their capacity to scavenge LPO radicals in a dose-dependent manner. Thus, the extract exerts its effective antioxidant ability to inhibit the oxidation process and thus, protecting the biological system. These data suggest the strong free radical quenching and antioxidant potential of *H. rosa-sinensis* against free radicals.

Table 1: Qualitative analysis of methanolic extract of *H. rosa-sinensis*

PHYTOCHEMICALS	RESULT
Flavonoids	++
Alkaloids	+
Saponins	-
Tannins	++
Phenolic compounds	++ +
Terpenoids	++
Glycosides	-
Steroids	-

+++ =High;++ = Moderate; + = Low; = Absent

The capacity of the aqueous flower extract to scavenge these radicals suggests that it encompasses phytochemicals that act as electron donors and can interact with free radicals to transform them into more stable products and trigger a chain of radical reactions (Michalak, 2022). There is a well-established positive connection between the total phenolics and flavonoids content with antioxidant activity (Cai *et al.*, 2004; Pan *et al.*, 2007). The higher free radical scavenging activity in DPPH may be due to the presence of flavonoids, which contributes to the antioxidant system. The extract may help to reduce lipid peroxidation and quench free radicals, as it contains phenolic compounds with ring structures and carboxylic groups (Rice-evans *et al.*, 1995; Chidambaram *et al.*, 2019). It can be observed that, the quantified free radical scavenging activity showed different inhibition rates in these assays, which might be due to the environmental condition and distinct reaction of antioxidant components in the *H. rosa-sinensis* with the certain radicals or oxidants (Chaves *et al.*, 2020). Thus, *H. rosa-sinensis* were able to shield the membrane from free radicals, demonstrating its significance in offering health benefits.

Preliminary phytochemical screening:

Since ancient times, plant and animal-based substances have been harnessed for their therapeutic potential in the form of crude drugs, without isolating pure compounds. The constituents present in these crude drugs play a crucial role in determining their pharmacological effects. Due to the intricate composition of herbal drugs, it becomes imperative to identify the active constituents that contribute to their therapeutic effects (Nortjie *et al.*, 20220.

The preliminary qualitative phytochemical screening of crude extract of *Hibiscus rosa-sinensis* is summarized in Table 1 which showed the presence of flavonoids, alkaloids, tannins, phenols and terpenoids. According to reports, phytochemicals reduce the risk of oxidative stress-related degenerative diseases by inhibiting hydrolytic and oxidative enzymes, such as lipid peroxidation, thereby protecting the cell's constituents from harmful oxidative damage (Vinary *et al.*, 2010).

The presence of these kinds of phyto-constituents are responsible for the pharmaco-logical properties of medicinal plants. The qualitative analysis showed variations in the content of phytocompounds, which may be due to the geographical or physiological conditioning and species difference of the flower. There was an increase in phenolic compounds in our results, which may be due to the high extraction temperatures, solvent properties such as surface

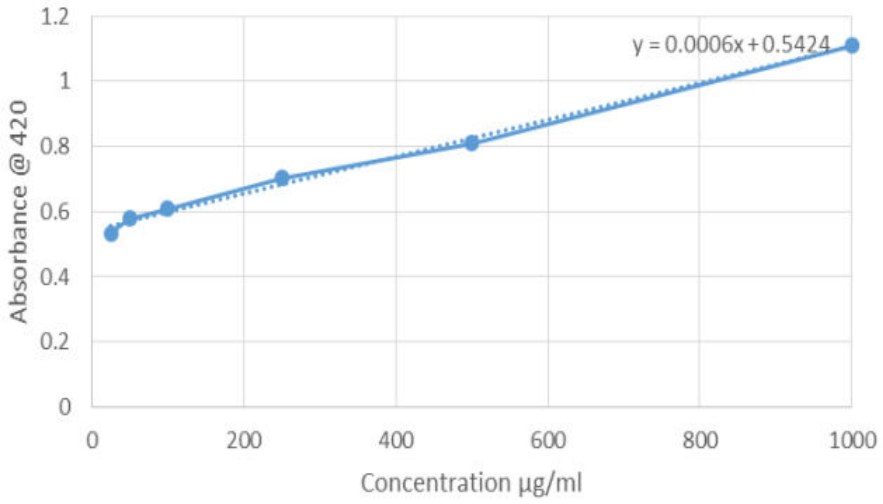


Fig. 4: Total Flavonoid-Quercetin standard curve.

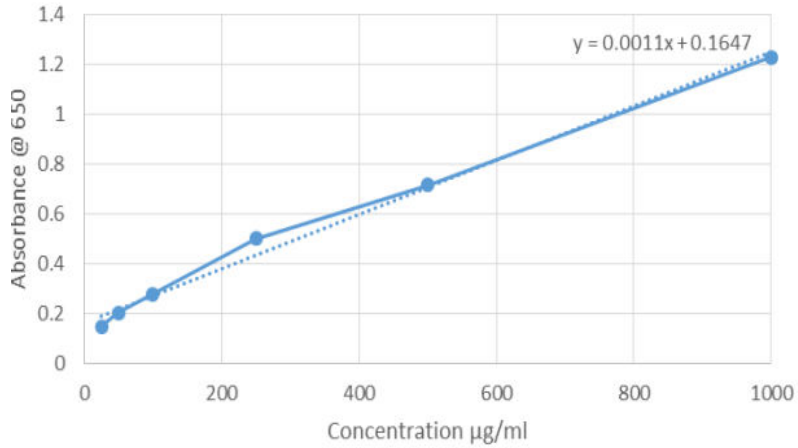


Fig. 5: Total Phenol-Gallic acid curve.

tension and viscosity, which influence the integrity of cell membranes and affect extraction dissolution and stabilization (Onyebuchi and Kavaz, 2020). Thus, *Hibiscus rosa-sinensis* contains a variety of biologically active secondary metabolites that may be used as a source of medications for herbal medicine.

Total phenolics, and flavonoids content:

Secondary metabolites known as phenolic compounds are greatly impacted by both hereditary and environmental influences. The amount of concentration varies based on their species diversity and plant parts. The quantitative analysis results exhibited the total amount of phenolic contents to be 713.90 µg/ml or 0.713 mg of GAE for 1 mg of the extract using standard graph (Fig. 4). The total flavonoid content was found to be 234.33 µg/2mg or 0.117 mg of Quercetin for 1 mg of the extract (Fig. 5).

There are many bioactivities associated with phenolic compounds, including antioxidants, anticarcinogenic, anti-mutagenic, and anti-inflammatory properties. In addition, phenolic substances disrupt signalling pathways that lead to apoptosis and reduce cell migration, proliferation, and metabolism during the development of cancer (Sharifi-Rad *et al.*, 2023).

Flavonoids, being diverse plant pigments, are considered potentially safe and non-immunogenic drugs. Due to their small organic compound nature, they have been traditionally absorbed by the human body. Flavonoids, like kaempferol and quercetin, act by altering membrane permeability and by inhibiting membrane bound enzymes, such as the ATPase and phospholipase A2 (Abou Baker, 2022). Molecular-level mechanistic studies have shed light on the wide range of biological properties by specific flavonoids, contributing to their potential beneficial effects on human health(Lee *et al.*, 2007).

Antifungal activity:

Fungal infections are currently challenging to treat due to a lack of available drugs, resistance to drugs, high costs, and toxicity. Consequently, antifungal agents need to be replaced with novel alternatives that are more effective (Vahedi-Shahandashti and Lass-Flörl, 2020). There are only few studies on antifungal activity of *Hibiscus sp.* Plants or microbial extracts can be evaluated for antimicrobial activity using the agar well diffusion method (Manandhar *et al.*, 2019).

The antifungal activity of the aqueous extract of *H. rosa-sinensis* was confirmed by the formation of zone of inhibition against selected fungal species *Rhizopus* and *Penicillium* along with standard drug fluconazole. The antifungal spectra against *Rhizopus* and *Penicillium* showed the maximum zone of inhibition at 1000 µg/disc. Based on the findings (Table 2, Fig. 6), the extract resulted in maximum zone of inhibition of 13 mm against the strain *Rhizopus,* while against *Penicillium,* the maximum zone of incubation was observed to be 15 mm. However, the extract did not exhibit any antifungal activity against *Rhizopus* at 100 µg/disc and 250 µg/disc and against *Penicillium* at 250 µg/disc concentration. Results obtained with these water extracts may be more advantageous for food and pharmaceutical applications than those obtained with other solvents. Hence, the aqueous extract of *H. rosa-sinensis* exhibited effective antifungal properties and could be assessed for further applications.

The activity may correspond to the presence of certain phytocompounds. A single test organism may respond differently to a tannin or flavonoid chemical isolated from different plant parts or origin. The inhibitory diameters (mm) could differ depending on how susceptible the organism is to the antifungal components in the extracts, and when the concentrations are changed, they might still have the same therapeutic potency (Gonelimali *et al.*, 2018). There were differences in the antifungal activity of the flower extracts, as evidenced by the inhibitory zones at which values were effective on the tested species. These variations in sensitivity may also be related to changes in the organism's growth rate, their dietary needs, the temperature, and the size of the inoculums (Davis *et al.*, 2005). The clinical spectrum of infections caused by *Rhizopus* and *Penicillium* spp. include Mucormycosis, sinusitis and pneumonia, hypersensitivity reaction and localized granuloma with dissemination being more common in patients with underlying illnesses. In addition to traumatic implantation, spore inhalation can lead to illness (Lyratzopoulos *et al.*, 2002; Wagner *et al.*, 2017). Plant extracts have the potential to provide new antimicrobial chemicals, particularly those that are effective against specific microbial infections. The aqueous treatment in this *in vitro* experiment demonstrated that they prevented fungal growth, however, the extent of their efficacy varied. The findings of the current study imply that these extracts include chemicals with antifungal capabilities that can be further investigated for its antimicrobial action. With regard to battling pathogenic microbes, this antifungal investigation of *H. rosa-sinensis* extracts showed the possibility of using it in the treatment of fungal infections.

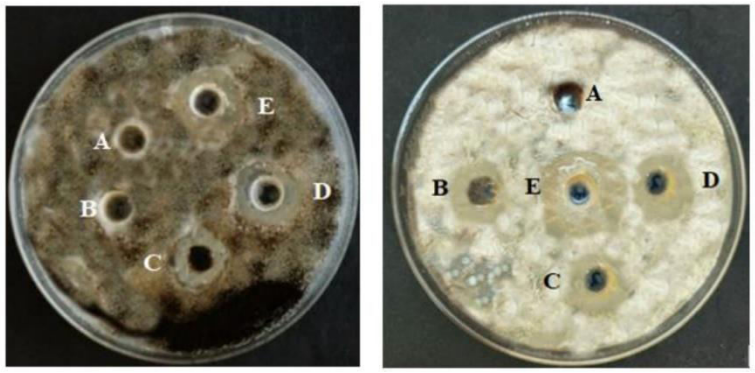
Cell viability:

In vitro cytotoxicity screening is an effective strategy to measure cell death caused by plant extracts or components to identify its antineoplastic properties. The MTT assay measures cell survival and mitochondrial function. It measures metabolic activity by measuring the

Table 2: Zone of Inhibition formed by *H. rosa-sinensis* against fungal strains

Zone inhibition in mm		
Concentration	*Rhizopus*	*Penicillium*
100 µg (A)	-	-
250 µg (B)	-	10
500 µg (C)	9	13
1000 µg (D)	13	15
Standard drug fluconazole (E)	16	17

(- denotes no zone of inhibition)



Rhizopus ***Penicillium***

Fig. 6: Antifungal activity of *H. rosa-sinensis.*

formation of purple formazan product when cells decompose tetrazolium salt (Igarashi and Miyazawa, 2001). The present study used the breast cancer cell line MCF-7 as the test system to examine more effective treatments for breast cancer, which is on the rise worldwide. MCF-7 breast cancer cells were treated with varying concentrations of aqueous flower extract in order to assess the cytotoxic behaviour of aqueous flower extract of *H. rosa-sinensis.* The findings (Fig. 7) showed that cell viability decreased in a concentration dependent manner with varying doses. At the highest tested concentration, approximately 70% of cells exhibited reduced viability. After 24 h of treatment, the IC_{50} concentration was determined to be 72.5 µg/ml. Microscopic observations demonstrated that upon exposure to lowest concentration of the flower extract (20 µg/ml), there were no observable morphological changes in MCF-7 cells. The number of deceased cells increased progressively with higher concentrations of the extract treatment. Additionally, at elevated extract concentrations, a noticeable enlargement of the cells was observed. Thus, aqueous flower extract had a remarkable impact on MCF-7 cells (Fig. 8). The MTT assay is employed to assess the cytotoxic potential of flower extract on MCF-7 cells, aiding in determining their effectiveness in inducing cytotoxic effects on ER+ breast cancer cells.

Cytotoxic assays play a significant role in the exploration of natural products for drug discovery. Understanding the mode of cell death is crucial in assessing the efficacy of cytotoxic agents (Hamsa *et al.*, 2022). Based on the activity, this extract appears to be effective against ER+ breast cancer cells and could be considered as a starting point for acquiring safe cytotoxic drugs. As evident from our phytochemical analysis, *H. rosa sinensis* contains diverse secondary metabolites that could contribute to its cytotoxic properties. The study findings imply that the extract of *Hibiscus rosa-*

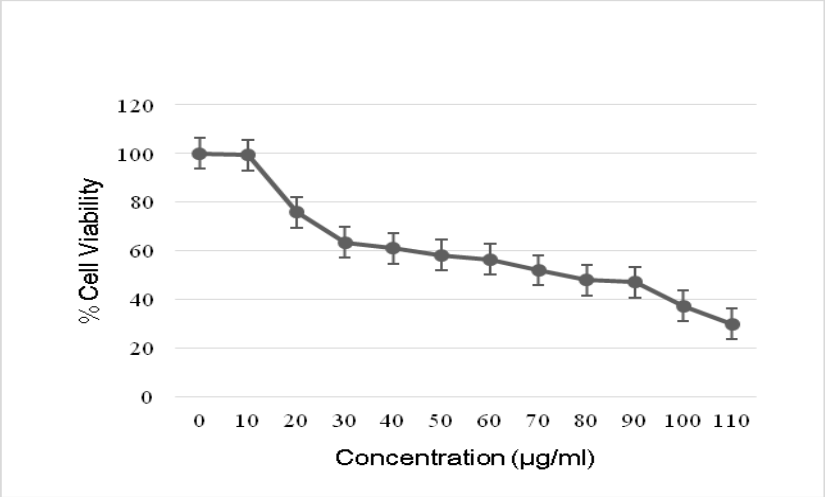
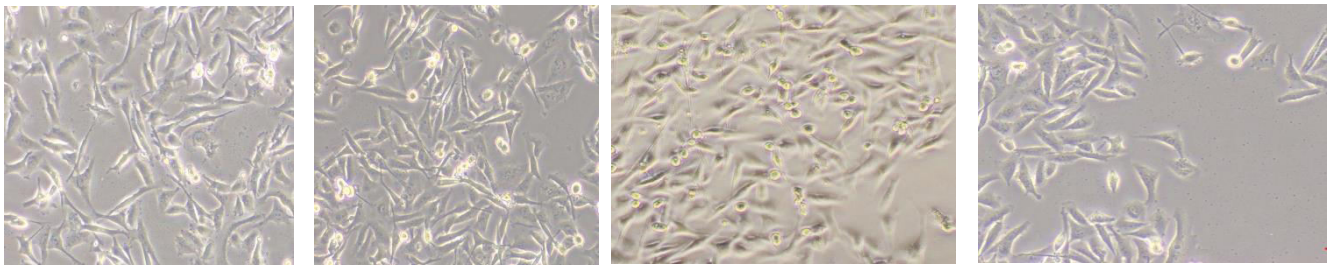


Fig. 7: Cell viability by *H. rosa-sinensis* by MTT assay.



Control **20 µg/ml** **72.5 µg/ml** **100 µg/ml**

Fig. 8: Morphological changes exhibited by *H. rosa-sinensis* extract on MCF-7.

sinensis might contain cytotoxic compounds capable of inducing cell death and inhibiting cancer cell proliferation, leading to potential therapeutic effects (Goldberg *et al.*, 2017).

Conclusion

This study highlights the significant role of phytopharmaceuticals in promoting health benefits and their potential as alternative therapeutic agents. *Hibiscus rosa-sinensis* flower extract has revealed promising pharmacological properties. The extract exhibited strong antioxidant activity, attributed to its rich content of secondary metabolites. Additionally, the extract demonstrated notable antifungal effects against *Rhizopus* and *Penicillium*, suggesting its potential in combating Candida-related infections. Moreover, the extract displayed potent cytotoxicity against MCF-7 breast cancer cells, indicating its promising role in cancer treatment. These findings emphasize the importance of further investigating the therapeutic potential of *Hibiscus rosa-sinensis* for the development of novel drugs and complementary healthcare approaches.

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