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# Innovations in STEAM: Research & Education

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## Prescribing Trends of Hepatitis C in Hospitals of Faisalabad, Pakistan

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### ABSTRACT

**Background:** Hepatitis is a priority area of public health in Pakistan in which Hepatitis B and C is prevalent at high prevalence and presents the enormous challenge for the healthcare sector.

**Objective:** To find out the trends, risk factors and control strategies for hepatitis in Pakistan.

**Methodology:** This study was conducted on patients who got treatment with hepatitis C virus (HCV) medicines; a comparative cross-sectional survey was done. To do this, 270 patients were given a standardised, closed ended questionnaire to fill out. Data were compared using age, gender, marital status, present medication and side effects. Epidemiology of hepatitis was studied on the basis of English and Urdu data from English and Urdu newspapers, national health databases, as recorded in hospital records and published papers. The chosen technique is in accordance with well-elaborated principles of public health research that guarantee precision and reliability. The results of the Pakistan Health Research Council, the Pakistan Bureau of Statistics and the World Health Organisation were used with the secondary data and carried out retrospective observational analysis based on the secondary data.

**Results:** Infection rates by species, geographic distribution and the success of prevention efforts in place are discussed based on the data given in the national health, research articles. It was seen that unsafe medical procedures, low vaccination coverages and low awareness on the part of the common person brought on a lack of burden of hepatitis, in particular, the rural populace and the individuals who were unfortunate. Changes have been made with government led initiatives including vaccination program and blood screen policies; however, the application is hindered by poor healthcare accessibility and socioeconomic barriers. The study gives an edge to the importance of public health interventions such as mass awareness campaigns, better sanitation and wider vaccination. Healthcare policies be strengthened and hepatitis control strategies be integrated into the primary healthcare services to achieve a great failure in reduction.

**Conclusion:** A multi-sectoral response that combines government agencies, healthcare providers, and community outreach needs to be employed to limit spread of hepatitis in Pakistan.

### INTRODUCTION

About 180 million individuals throughout the world have contracted hepatitis C virus (HCV). Various nations have stated prevalence rates of hepatitis C infection. According to Alberts *et al.* (2022), the rates of HCV were found to be highest in Asia, North Africa, and the Middle East. They were moderate in South Asia, Sub Saharan Africa, Central and Southern Latin America, the Caribbean, Oceania,

Australasia, Middle Eastern and Central Europe, and Tropical Latin America and North America. About 15–20% of those infected with HCV are able to recover, whereas the remaining 75–85% develop hepatitis C. Research shows that, compared to other cities in Pakistan, the prevalence of hepatitis C is on a rise in Faisalabad, where it affects roughly 24% population (Abbas *et al.* 2024). When it comes to liver disease, hepatitis C is at the top of the list for complications, deaths, and transplants. Only 1% of hepatitis



cases in Pakistan are treated (Khan *et al.* 2020). Uninformed people about the disease spread, high treatment costs, and a shortage of qualified medical personnel are reasons for rising hepatitis ratio and the dismal treatment rate (Proquest.com 2021).

Beginning in 1999 as a monotherapy, interferon (IFN) was later shown to be an effective in combination with ribavirin (RBV) in 2001. As of 2004, the gold standard for treatment was the introduction of RBV in conjunction with PEGylated interferon alpha. Significant adverse effects of IFN prevented it from providing the desired therapeutic benefit, which led to discontinuation of its usage (1-6SI). The issue of IFN toxicity persisted even when the dosage was reduced. Myalgias, stomach pains, sleeplessness, anorexia, and fever were the first noted adverse effects. Infections of the urinary tract, the lungs, the brain, hyperthyroidism, hypothyroidism, psychosis, and suppression of the bone marrow were among the adverse effects noted with long-term usage (Salari *et al.* 2022).

From 2001 to 2011, IFN and RBV were the gold standard drugs for treating HCV. However, several direct acting antiviral (DAA) medication combinations were authorised in 2015 by the European Medicines Agency (EMA) and the Food and Drug Administration (FDA), and these combinations exhibited improved infection treatment response with a decreased risk of side effects. The use of antivirals with direct action has a remarkable positive impact on patient's health while dealing with HCV. According to Naz and Asghar (2023), there are a number of viral protein inhibitors that may aid in the removal of HCV infection from biological systems. These include NS3/4A protease, NS5A, and NS5B polymerase.

Chills, sores, ulcers, pale skin, altered behaviour, lightheadedness, sleeplessness, difficulty breathing, headache, nausea, allergic reactions, constipation, fever, and body aches are some of the antiviral adverse effects that have been seen. Hepatitis C infection spreads via the blood. A chronic infection may develop after an acute infection of HCV. In Faisalabad, where hepatitis affects at least 24% of the population, it is becoming an increasingly pressing issue. The liver may develop cirrhosis and hepatocellular cancer as a result of an HCV infection. Thus, it is crucial to diagnose and treat at an early stage (Guntipalli *et al.* 2021; Kamili and Wester 2024).

The cost of controlling hepatitis is too high all over the world (Lim *et al.* 2021). Despite that it is important to invest in this public welfare program. This study was an attempt to describe and analyze the hepatitis C pharmaceutical therapy prescription pattern in Faisalabad, Pakistan to determine the efficacy of the therapy and patient satisfaction grade of the prescribed drugs.

## MATERIALS AND METHODS

A comparative cross-sectional survey was done on patients who got treatment with HCV medicines. To do this, 270

patients were given a standardised, closed ended questionnaire to fill out. Data was compared using age, gender, marital status, present medication and side effects. Epidemiology of hepatitis was studied on the basis of data from English and Urdu newspapers, national health databases, as recorded in hospital records and published papers. The chosen technique is in accordance with well-elaborated principles of public health research that guarantee precision and reliability. The results of the Pakistan Health Research Council, the Pakistan Bureau of Statistics and the World Health Organisation (WHO) were used with the secondary data and carried out retrospective observational analysis based on the secondary data. It is also based on a review of patient data from a tertiary healthcare institution hospital to provide trends in prevalence. In the analysis of the Hepatitis A, B, C, D and E, infection patterns from 2000 to 2020 (Ullah *et al.* 2022) are presented.

All reported viral hepatitis cases in Pakistan in that period have been analysed in the present study. It includes all dimensions of gender, age and socioeconomic status, and urban and rural residents. There was no study, and when the diagnostic criteria were inconsistent, there were duplicate records. All the strains of hepatitis incidence, prevalence and mortality rates are calculated with the help of SPSS V.17. Descriptive statistics included frequency distribution, standard deviations and means (Falak *et al.* 2020). So, we worked forwards after trending the time series and tried to find a relationship between demographic factors and illness prevalence with Chi square test. In their study, Moradi *et al.* (2020) state that  $P < 0.05$  is statistically significant in this regard.

Patients were not required to provide their permission for this research since it made use of already-public data extracted from an anonymised medical record. Following standard procedures for research ethics and participant confidentiality, the consent from Institutional Review Board of concerned hospitals was obtained to conduct the study before the individuals were enrolled.

From around four months in the beginning of 2019, all of Faisalabad's hospitals were included in the research. We included all HCV patients who were diagnosed without age discrimination and were currently taking treatment for their HCV. The University of Faisalabad's ethics council gave approval for this study. This is because, in accordance with the Declaration of Helsinki Principles, all patients and legal guardians were required to provide written informed permission before they could participate in the trial (Ali 2023). One hundred thirty-seven patients at Allied Hospital, Liver Centre, District Headquarter Faisalabad, Social Security Hospital, and Aziz Fatima Hospital, Faisalabad, who were given medication for HCV infection, filled out a questionnaire created in accordance with national standard treatment guidelines for hepatitis (Saleem *et al.* 2022).

Once several hospital coordinators gave permission, we selected a location for the patient interviews. The completion of the questionnaire was not done in the

presence of any medical professional. Medication, administration method, side effects, treatment satisfaction, treatment cost-effectiveness and diagnostic tests were all included in the questionnaire. Also, details were gathered on the patients age, gender, marital status, occupation, level of education and familiarity with the risk variables (Fung *et al.* 2009; Hashmi *et al.* 2021).

To compare quantitative variables, we used Student's *t*-test, which takes the mean and standard deviation into account. The comparison was considered significant at  $P < 0.05$ . Data were analyzed using SPSS V.17.

## RESULTS

Of the 270 patients participating in research, a lesser number of patients ( $n=99$ ) were under the age of 40, while the vast majority (63.3%,  $n=171$ ) were older than 40 years (Table 1). Out of 270 patients, 156 were female (57.2%) and 114 were male (42.2%) (Table 1). With  $P < 0.001$ , 91.5% of the individuals ( $n=247$ ) were married, while 8.5% ( $n=23$ ) were never married. (Table 1).

The occupations represented among the 270 patients were: 124 (45.5%) were housewives, 15 (5.6%) were government employees, 13 (4.8%) were farmers, 17 (6.3%) were businessmen, 29 (10.7%) were teachers, 3 (1.1%) were mill employees, 2 (0.7%) were bankers, 6 (2.2%) were wholesalers, 51 (18.9%) were labourers, and 10 (3.7%) were students (Fig. 1). Of the patients surveyed, 100 (37% of the total) gave a positive response when asked about a family history of hepatitis C, whereas 170 (67%) did not ( $P < 0.001$ ) (Fig. 2).

Among DAAs, Sofosbuvir was used by 24.8% of the participants, Daclatasvir by 24.4%, Entecavir by 0.4%, Acyclovir by 0.7%, RBV by 7.8%, and PEGylated alpha2b by 1.5%. The most common combinations used were Velpatasvir and Sofosbuvir (38.9% of all prescriptions) and IFN and RBV (1.5% each). Patients older than 40 years were more likely to use a combination therapy (34.1% vs. 8.8% for patients younger than 40) (Fig. 3). Sofosbuvir (24.8%), Daclatasvir (24.4%), RBV (7.8%), and PEGylated alpha-2b (1.5%) were the antiviral medications most often administered. Together, Velpatasvir and Sofosbuvir accounted for 38.9% of all combination therapies, with IFN and RBV coming in at 1.5% (Fig. 4). A high cost of HCV therapy was mentioned by 233 patients (86.3%). Among all patients, just 37 (13.7%;  $P < 0.001$ ) were exempt (Fig. 4).

Among 270 patients prescribed HCV medication, 167 (61%) were on it for 1–6 months, 38 (14.1%) for 6 months to a year, 28 (10.4%) for 1–2 years, 18 (6.7%) for 2–4 years, 8 (3%) for 4–5 years, and 11 (4.1%) for 5–10 years (Fig. 5). Although 5.6% of patients were dissatisfied, 94.4% were satisfied with their drug treatment (Fig. 6).

In patients receiving antiviral treatment, the following adverse events were noticed most frequently: weakness (19.6%), constipation (20.0%), dizziness (12.6%), fever

(9.6%), and sleeplessness (9.6%). Tolerance responses (4.8%), nausea (5.2%), headache (4.4%), and bodyaches (8.5%) were the mild adverse events. Shortness of breath (1.1%), pale complexion (1.9%), chills (2.2%), and blisters (0.4%) were minor adverse events (Fig. 7).

There are a number of comorbid illnesses that might aggravate HCV infections. Comorbidities were seen in 160 out of 270 individuals. Hypertension accounted for in 70 patients (58.5%), diabetes in 58 patients (25.9%), and anaemia in 32 patients (11.9%) among the top ranking conditions. No comorbidities were found in 110 individuals (Table 2).

## DISCUSSION

Consistent with other research showing age-related susceptibility owing to protracted exposure to risk factors, such as risky medical procedures and blood transfusions, the study shows a significant incidence of HCV in Faisalabad, especially among those over 40 years old. Existing research suggests intra-household transmission, possibly via shared hygiene items or close contact, and the much higher infection incidence in married persons supports this idea (Farooq *et al.* 2024).

According to Younas *et al.* (2021), patients older than 40 years were more likely to have combination treatment. The most widely given combination, with a prevalence of 38.9%, was Velpatasvir + Sofosbuvir (Fig. 1), which shows a move away from IFN-based therapy and towards DAAs. Because of their greater effectiveness and reduced side-effect profile, DAAs are recommended in worldwide treatment recommendations (Curry *et al.* 2015). Still, a huge problem is the lack of accessibility; 86.3% of patients indicated high cost of medicine as a big obstacle. This is in line with Hill *et al.* (2019) who found that high cost was the greatest obstacle to treatment, especially in low-income nations.

A high prevalence of comorbidities like diabetes and hypertension among HCV patients lends credence to earlier studies that established a connection between long-term HCV infection and metabolic diseases as well as cardiovascular problems (Bozkurt *et al.* 2016; Petrie *et al.* 2018; Nasrallah *et al.* 2024). Based on these results, healthcare providers should work together to treat HCV and any co-occurring diseases (Tariq *et al.* 2023).

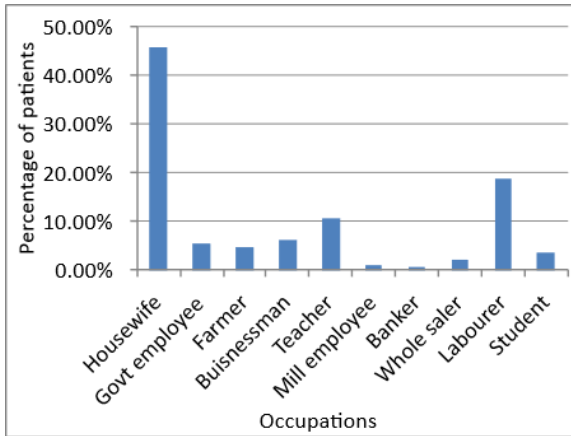
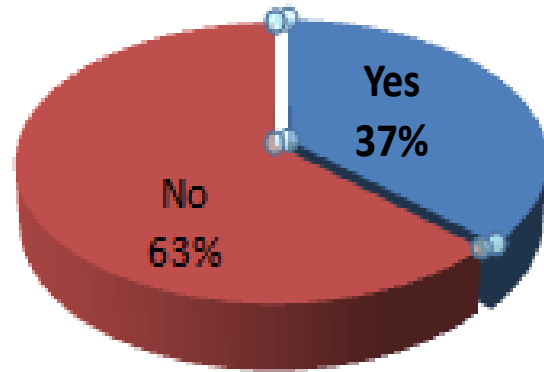
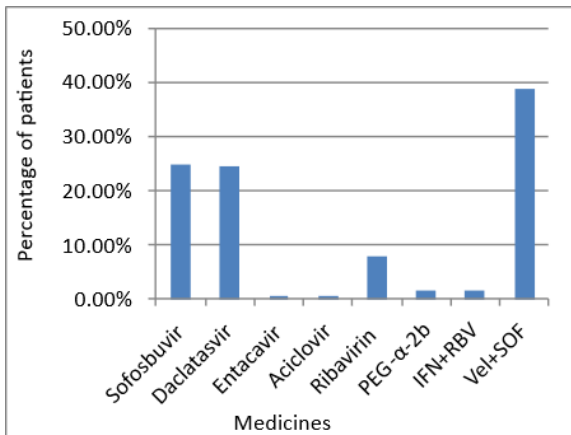
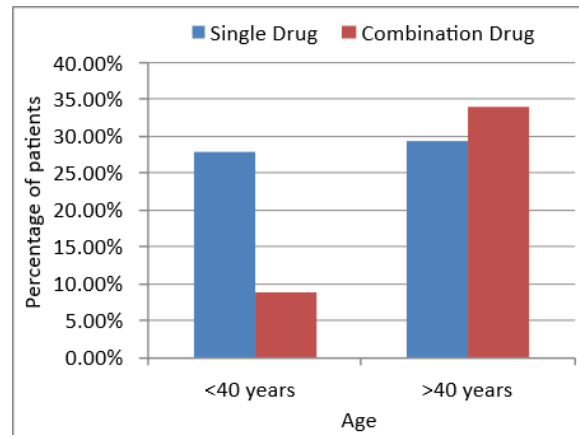
Majority of the patients were satisfied with their treatments (94.4%), although a small percentage had side effects, including fatigue (19.6%), constipation (20%) and vertigo (12.6%). These results are somewhat different from those of Western populations, where reports of exhaustion and gastrointestinal problems were more prevalent, which may indicate that medication tolerance and patient-reported outcomes vary by geography (Bibi *et al.* 2023; Marcellin *et al.* 2023).

The current research confirmed that married people had a greater HCV prevalence. Factors like sex and sharing



**Table 1:** Demographic data of the patients

Category	Frequency	Percentage data	P-value
Age			
• <40 years	99 /270	36.7%	<0.001
• >40 years	171/270	63.3%	
Gender			
• Male	114	42.2%	<0.001
• Female	156	57.8%	
Marital status			
• Married	247	91%	<0.001
• Single	23	8.5%	


**Fig. 1:** Occupational distribution among the patients for the prevalence of HCV

**Fig. 2:** Distribution of patients with respect to family history of HCV

**Fig. 3:** Trend of DAAs prescription to the HCV patients

**Fig. 4:** Trend of using drugs combination among age groups

housing may contribute to the transmission of HCV from one partner to the other. Prior research by Osmond *et al.* (1993) and Piazza *et al.* (1997) provided evidence that sex may transmit HCV. The discovery of HCV RNA in saliva lends credence to the idea that the virus could have spread through non-sexual and non-parental household transmission (Ackerman *et al.* 2000).

Among the individuals in this research, females were more affected by HCV than males. according to research by Sood *et al.* (2018), out of a sample size of

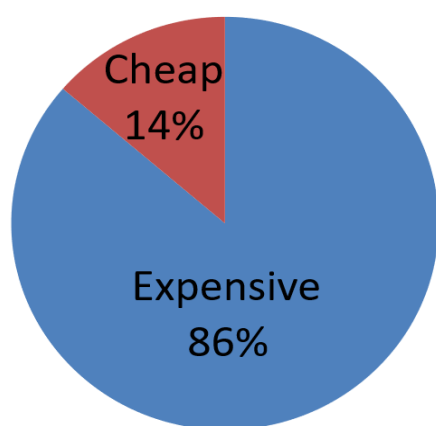
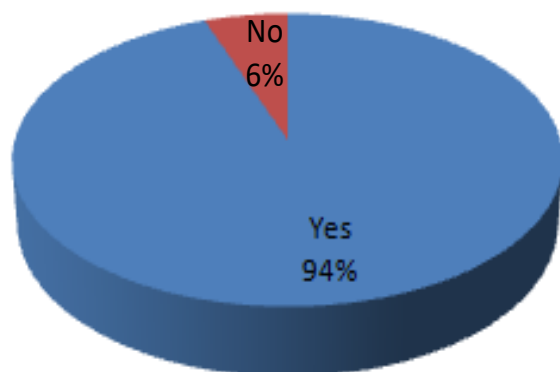
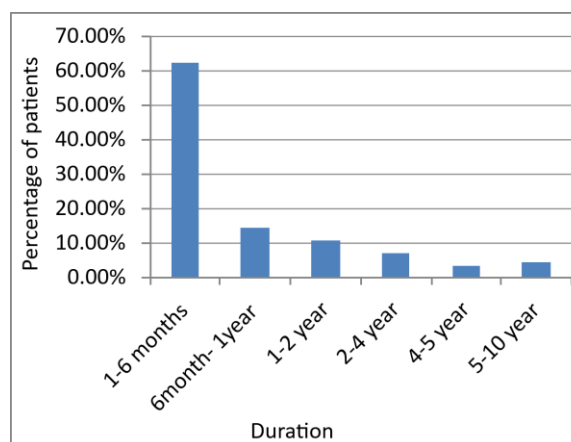
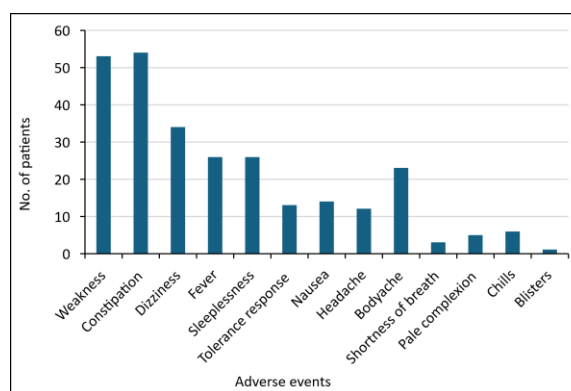
5543 patients, females had a greater prevalence of HCV (53.8%) than males (46.2%), that had 5543 patients. Contrarily, Mahmood *et al.* (2021) indicated a greater prevalence of HCV in males (67.14%) compared to females (32.85%).

This research confirms the findings of Butt *et al.* (2007) and Younossi *et al.* (2013) that demonstrated the greatest rate of co-morbidity between HCV and hypertension. The risk of coronary artery disease (CAD) was greater in HCV infected individuals compared to HCV



**Table 2:** Comparison for occurrence of comorbidity among the patients

Variables	No. of patients	Percentage	P value
Hypertension	70	58.5%	<0.001
Diabetes	58	25.9%	<0.001
Anemia	32	11.9%	<0.001
No disease	110	40.7%	<0.001

**Fig. 5:** Opinion of patients with respect to cost of the medicines**Fig. 7:** Level of satisfaction of patients with the DAAs for the treatment of HCV**Fig. 6:** Duration of use of HCV medication by the patients**Fig. 8:** Adverse events experienced by patients with the use of HCV drugs during treatment

uninfected patients, according to Butt *et al.* (2007) A high prevalence of congestive heart failure (CHF) was observed in patients infected with HCV (Younossi *et al.* 2013). Völzke *et al.* (2004) on the other hand, could not detect a correlation between HCV and CVD. Our findings corroborate those of Sir Ganga Ram Hospital Karachi, where researchers found that people with diabetes, particularly type 2 diabetes mellitus, had an increased chance of contracting HCV infection (Guo *et al.* 2013). Additionally, this research found that HCV and anaemia often occur together. Smith *et al.* (1998) found that Homeostatic Iron Regulator gene mutations are more common in HCV patients and are linked to higher iron storage in HCV patients. On the other hand, Thorburn *et al.* (2002) found no evidence that HFE mutation contributed to

iron buildup in liver biopsies taken from HCV infected individuals.

The current research demonstrated that Velpatasvir and Sofosbuvir were often used together. Curry *et al.* (2015) and Feld *et al.* (2015) showed that this combination is quite successful, with SVR rates ranging from 98–99% after 12 weeks of therapy. Additionally, our research found that antiviral medication for hepatitis C often comes with side consequences. In contrast to our findings, the previous study found that 47.6% of patients experienced fatigue, 38.1% experienced GIT disturbance, 14.3% had headaches, and only 2 patients experienced dyspnoea (Mehmood *et al.* 2019).

Eighty percent of HCV-positive individuals should be treated by 2030, according to the WHO (Raja *et al.* 2020). A

high cost of medication therapy is a major barrier to treatment in many nations. High cost of hepatitis C medicines was a concern for 86.3% of patients (Fig. 2). In their 2016 study, Hill *et al.* (2019) found that current prices for Sofosbuvir for a 12-week therapy range from \$84,000 in the United States to \$53,400 in the United Kingdom, \$46,139 in France, \$27921 in Spain and Portugal, \$7,000 in Brazil, and \$483 in India. Siddique *et al.* (2020) estimated that cost of a 12-week course of generic Sofosbuvir would be ~\$150 USD.

The current investigation confirmed the prevalence of side effects associated with hepatitis C antiviral treatment. We found that 47.6% of patients had tiredness, 38.1% had GIT disruption, 14.3% had headaches, and just 2 individuals had dyspnoea.

In order to increase early detection and accessibility of HCV medicines in Pakistan, this research highlights the immediate need for treatment techniques that are both cost-effective and part of better public health programs. Expanding screening programs and assessing cost-effective generics should be the primary goals of future research aimed at reducing disease burden. Our research showed that hepatitis C was more common in people over the age of 40 compared to younger people under the age of 40. This finding is in line with what Sood A *et al.* found in their study, which also found that the prevalence of HCV increases with age (Abbas *et al.* 2024; Kashif *et al.* 2024).

## CONCLUSIONS

Lack of continuous medical care and the expense of therapy are big obstacles to eradication HCV. There has been clear progress in treating HCV infection with minimal side effects and significant clinical efficacy in recent years. However, the enthusiasm of IFN-free DAAs has been muted by very high cost of drugs. Majority of the recently established regimens are expensive, making their broad use impractical. Pakistan needs to make hepatitis C treatment and prevention a top priority because the infection affects ~5% Pakistanis. Prevalence of hepatitis C was highest among married people in Faisalabad, and that the disease disproportionately affects those who are over 40 years of age. Velpatasvir + Sofosbuvir is the most frequently prescribed combination as DAAs, which has improved treatment efficacy. A multidisciplinary approach to healthcare is necessary due to frequent occurrence of comorbid conditions like hypertension and diabetes. There should be more government intervention to subsidise antiviral treatments, raise awareness about HCV infections and contacts, and expand screening programs. Long-term efficacy of inexpensive generic DAAs needs to be studied, and ways to incorporate HCV management into primary health care be explored. Improved healthcare infrastructure and universal treatment access are essential to achieve the WHO's goal of eradicating hepatitis C as a public health threat by 2030.

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## DATA AVAILABILITY

The data will be made available upon request to the author

## ETHICS APPROVAL

This paper is not relevant

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## Color Vision in Diabetic and Non-Diabetic Retinopathy Patients

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### ABSTRACT

**Background:** Diabetes (DM) is a widespread and significant health concern that can lead to various complications. Among its many effects, ocular problems are among the most prevalent and rapidly growing causes of morbidity worldwide.

**Objective:** To evaluate the color function in diabetics and non-diabetics.

**Methodology:** The study was cross-sectional type and carried out at the Madina Teaching Hospital Faisalabad. Total 58 participants, male and female, aged 35 to 75 years selected using a convenient sampling method. The study took place from September 2018 to February 2019. Color vision was assessed using the conventional Farnsworth D15 test. Retinopathy severity was evaluated with a slit lamp and a +70D lens, following proper patient consent. A comprehensive medical, surgical, ocular, and drug history was taken for each participant. Each pseudophakic eye was tested monocularly, with each participant undergoing the test three times.

**Results:** A significant correlation was found between color vision impairment and pseudophakic diabetic patients ( $p < 0.05$ ), using Pearson's Chi-Square test. The mean score for color vision using the Farnsworth D15 test was  $2.17 \pm 1.05$ , while the pseudophakic group showed a mean score of  $1.05 \pm 0.116$ .

**Conclusion:** Diabetic patients with pseudophakia exhibit color vision defects, particularly affecting the blue axis. Color vision defects are more prevalent in patients with uncontrolled diabetes compared to those with controlled diabetes. Additionally, the severity of color vision defects tends to increase as the retinopathy progresses.

### INTRODUCTION

Diabetes (DM) is a widespread and significant health concern that can lead to various complications (Papatheodorou *et al.* 2018). Among its many effects, ocular problems are among the most prevalent and rapidly growing causes of morbidity worldwide. Even after cataract surgery with intraocular lens (IOL) implantation, vision can still be affected in diabetic patients, and the progression of retinopathy may worsen as a result of the surgical procedure (Sayin *et al.* 2015).

Diabetes is a complex disorder in which the body may not produce enough insulin or do not effectively use the insulin it produces. Insulin is vital for regulating blood sugar levels and ensuring that glucose is transported into cells for energy. The two main types of diabetes are Type 1 and Type 2, which were previously referred to as insulin-dependent and non-insulin-dependent, or juvenile onset and adult onset, respectively (Taware 2012).

Globally, over 285 million people are diabetic. The utmost difficulty is diabetic retinopathy; this is because of harm to the blood vessels within the retina because of diabetes-caused microangiopathy. In addition to retinopathy, diabetes can result in cataracts, glaucoma, nephropathy, and neuropathy (Krepler *et al.* 2002). The risk of growing retinopathy will increase with age and is similarly enhanced by poorly controlled blood sugar degrees, high blood pressure, excessive cholesterol, and relatives records of intense diabetic retinopathy (Kelkar *et al.* 2018). Color vision testing is a powerful way to know about the status of retinal damage. In diabetic people, visual function can be considerably altered depending on the severity of the disorder. Color vision defects are often an early sign of retinopathy and continues changes in color interpretations of patient can precede declines in visual acuity. Color deficiencies also can be as a result of other situations, together with glaucoma, macular degeneration, Alzheimer's disorder,





Parkinson's disease, and even chronic alcohol use (Male *et al.* 2022). Studies have proven that the outcomes of cataract surgical treatment are frequently worse in diabetic sufferers, especially people with diabetic retinopathy (Hwang *et al.* 2015). High glucose levels can cause the development of cataracts through glycosylation procedures, contributing to ocular complications (Hassan *et al.* 2010).

## MATERIALS AND METHODS

A cross-sectional study was conducted at the Ophthalmology Department of Madinah Teaching Hospital, Faisalabad. Total 58 participants, both male and female, were enrolled in the study using a convenient sampling method. The study was carried out between September 2018 and February 2019. Pseudophakic diabetic patients and without diabetes were included in the study, with both Type 1 and Type 2 diabetes patients aged 35–75 years, with reliable mental and systemic health.

Exclusion criteria included patients with a history of laser treatment, ocular conditions that might alter color vision, systemic diseases unrelated to diabetes, intraocular pressure more than normal ranges, posterior capsule opacities, clinically significant macular edema, and proliferative retinopathy.

The conventional Farnsworth D-15 color vision test was used to assess color perception. In this test, participants were asked to arrange 15 color caps according to hue, which were placed randomly on a white background. The test was performed in a well-lit room at a distance of 50 cm, both monocularly and binocularly, and each subject was tested three times. To assess the severity of retinopathy, a slit-lamp examination and +70D lens were used after obtaining informed consent from patients. Data was analyzed using SPSS software version 20, and the association between color vision defects and pseudophakic diabetic patients with background retinopathy was evaluated using Pearson's Chi-Square test. Ethical approval was obtained prior to conducting the study.

## RESULTS

About 58 participants were enrolled. Depending on age criteria two groups formed. The distribution of color vision defects in the group was calculated, with the most common color defect being blue (61%), followed by red-green defects (10.2%). The mean score for color vision on the D-15 test was  $2.17 \pm 1.05$  for the general population, whereas the pseudophakic diabetic group had a mean score of  $1.05 \pm 0.116$ . A Chi-Square test was used to assess the relationship between color vision defects and pseudophakic diabetic patients, with  $P = 0.05$  indicating statistical significance. This result suggests that color vision declines as diabetes progresses. The most common color vision defect in patients with retinopathy was observed in the blue axis, rather than the red-green axis (Fig. 1).

In the study, we categorized participants into two age groups: Group 1 (35–55 years) and Group 2 (56–75 years). In Group 1, there were 7 non-diabetic individuals, 10 with Type 2 diabetes, and 1 with Type 1 diabetes. In Group 2, there were 14 non-diabetic participants, 1 with Type 2 diabetes, and 10 with Type 1 diabetes. Notably, Type 2 diabetes was more prevalent in older age group (Fig. 2).

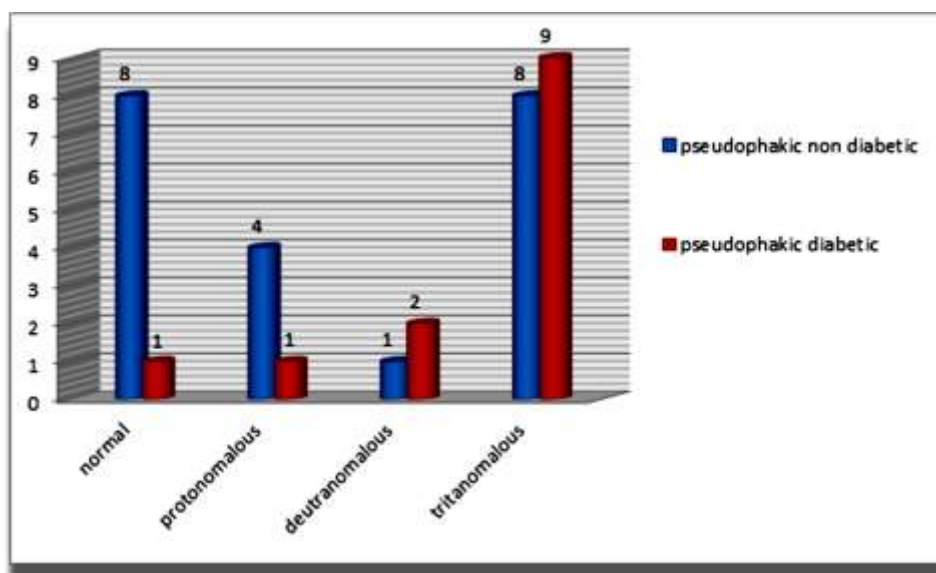
## DISCUSSION

A previous study by Gella *et al.* (2015) explored the impact of cataract surgery on color vision in both diabetic and non-diabetic pseudophakes. The study found that color vision declined after cataract surgery in both groups, but the severity of the decline was more pronounced in diabetic individuals. In this study, 22 diabetic pseudophakes with no retinopathy, 23 with background retinopathy, and 34 non-diabetic pseudophakes were examined. The results revealed that red-green perception sensitivity was significantly worse in the diabetic pseudophakes (normal versus retinopathy:  $P = 0.057$ ), with tritan discrimination sensitivity being worse in those with retinopathy (Gella *et al.* 2015).

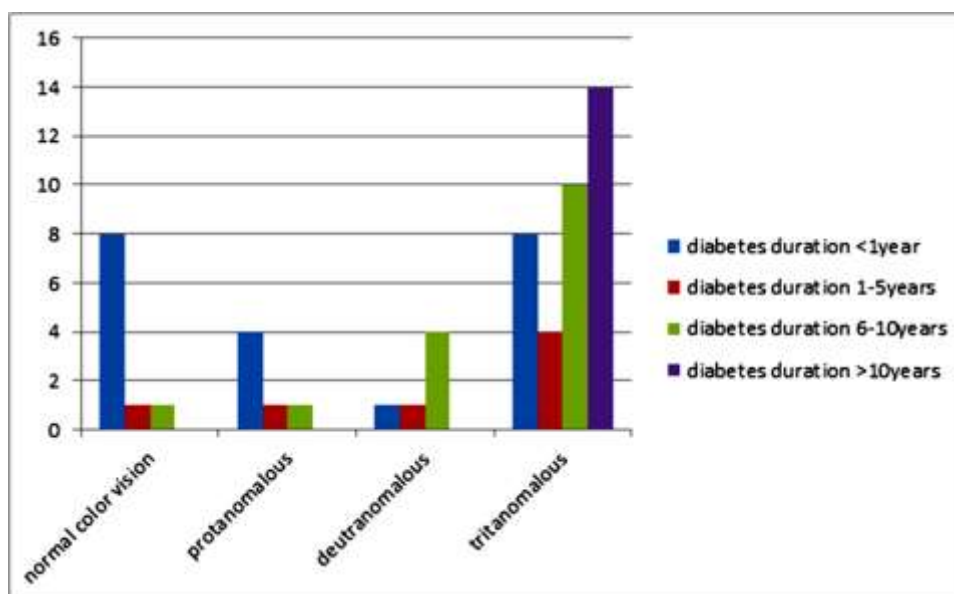
In another study conducted by Gella *et al.* (2017), color vision defects were examined in 21 diabetic patients (16 with insulin-dependent diabetes mellitus, IDDM, and 5 with non-insulin-dependent diabetes mellitus, NIDDM) and 19 non-diabetic individuals. All subjects had undergone cataract surgery, and their color vision was tested using the Farnsworth-Munsell 100 hue test. Diabetic patients had significantly higher error scores, particularly in the tritan axis ( $P = 0.02$ ). The study also excluded participants with secondary cataracts, glaucoma, or diabetic macular edema, as these conditions specifically affect foveal vision.

Our study aimed to evaluate color vision in pseudophakic diabetics with background retinopathy in the age group 35–75 years. A total of 58 participants were included, divided into two groups: Group 1 (35–55 years,  $n=20$ ) and Group 2 (56–75 years,  $n=38$ ). The study included 26 males and 32 females, with 21 non-diabetic individuals, 13 IDDM diabetics, and 24 NIDDM diabetics. NIDDM diabetes was more prevalent (40.68%) compared to IDDM (22.03%). Females had a higher prevalence of diabetes than males.

Visual acuity for all participants was measured, and those with clinically significant macular edema (CMO) or pre-proliferative/proliferative retinopathy were excluded from the study. Color vision impairment was most pronounced along the tritan axis, particularly in diabetic pseudophakes with background retinopathy. A significant association was found between color vision defects and the presence of background retinopathy ( $P = 0.05$ ). The distribution of color vision defects in the study population was as follows: 16.9% with normal color vision, 10.2% with red-green defects, and 61% with tritan defects. Tritan discrimination sensitivity changes were determinant for figuring out patients liable to excessive retinopathy. The



**Fig. 1:** A bar graph illustrating the presence and absence of color vision defects in pseudophakic diabetic patients with background retinopathy, showing normal color vision, protanomalous, deutanomalous, and tritanomalous conditions.



**Fig. 2:** Bar chart for relation of increase color defects with diabetes duration.

poorer color changes following cataract surgical operation in diabetic pseudophakes can be due to extended short-wavelength transmission thru the intraocular lenses, that could lead to retinal damage. This is mainly true inside the case of phacoemulsification, in which there's a shorter period of exposure to radiation all through lens removal, probably aggravating retinal harm.

## CONCLUSIONS

There was great effect on color vision for pseudophakic

diabetics. The color vision declines more seriously alongside the tritan axis, particularly in patients with retinopathy. These consequences spotlight the importance of incorporating color vision evaluation as an everyday part of pre-operative and submit-operative tests for diabetic sufferers encountering the process of cataract surgical procedure. Early detection of color impairment could assist within the management and tracking of diabetic retinopathy.

## AUTHOR CONTRIBUTIONS

NG: Concept, data collection, write up; RS: Formatting

## CONFLICTS OF INTEREST

The authors affirm that they possess no conflicts of interest.

## DATA AVAILABILITY

The data will be made available on a fair request to the corresponding author

## ETHICS APPROVAL

Approved by Institutional Ethical Review Board at its meeting held on May 23, 2019.

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## Phytochemical Constituents in *Putranjiva roxburghii* Seed Extracts with Potential Health Benefits

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### ABSTRACT

**Background:** *Putranjiva roxburghii* Wall. is an underutilized but valuable plant. It plays significant role in the traditional Ayurvedic and Unani systems.

Antioxidants are crucial in preventing the formation of reactive oxygen species (ROS), neutralizing existing ROS, and repairing damage caused by ROS.

**Objectives:** The objective of the study was to investigate phytochemical profiling of seed extracts of *P. roxburghii*.

**Methodology:** The seeds of plant were used for extraction with various solvents and the phytochemical screening and analysis including total phenolic contents (TPC), total flavonoid contents (TFC) and DDPH radical scavenging assay were carried out by standard methodologies. MS Excel was used to calculate the means, and standard deviation (SD) and to make graphs.

**Results:** The various extracts of *P. roxburghii* seeds showed the presence of phenols, flavonoids, saponins, alkaloids, carbohydrates sterols and terpenoids whereas aqueous extract did not show terpenoids. The glycosides and tannins were also absent in all these extracts. The mean value of TPC and TFC of methanolic, hydroethanolic and water extracts of seeds of *P. roxburghii* vary from 48.8 to 83.0 mg/g GAE and 565 to 915 mg/10 g CE, respectively. The mean  $\pm$  SD of DPPH % inhibition of methanolic, hydroethanolic and water extracts of seeds of *P. roxburghii* was  $69.2 \pm 8.9$ ,  $32.8 \pm 1.86$  and  $35.6 \pm 9.60$ , respectively.

**Conclusion:** The phytochemical screening of *P. roxburghii* seeds showed the presence of all representative groups, except tannins and glycosides in all extracts and terpenoids in aqueous extract. The hydro ethanolic extract of *P. roxburghii* seeds contain high contents of both TPC and TFC while methanolic extract showed highest DPPH inhibition.

## INTRODUCTION

Plants of medicinal values have been used to cure many diseases since ancient times. Several studies indicate that many medicinal plants exhibit antioxidant properties (Saeed *et al.* 2012). Antioxidants are crucial in preventing the formation of reactive oxygen species (ROS), neutralizing existing ROS, and repairing damage caused by ROS (Ighodaro and Akinloye 2018). *Putranjiva roxburghii* Wall is an underutilized but valuable plant (Emasushan and John Britto 2018). It has effective medicinal value and plays a significant role in the traditional Ayurvedic and Unani

systems (Gupta 2016; Pandey and Flulara 2022). Various parts of *P. roxburghii* are used for the treatment of different diseases (Unnikrishnan *et al.* 2015; Mradu 2016). It has an anti-inflammatory, antipyretic, analgesic, and anti-rheumatic herb, that is also useful to treat gynaecological and fertility disorders (Naik *et al.* 2023; Pandey and Flulara 2022). It is used to treat many diseases such as treatment of mouth and stomach ulcers, hot swellings, smallpox, burning sensation, ophthalmopathy and liver diseases (Mishra *et al.* 2021; Pandey and Flulara 2022).

Among the attributes of *P. roxburghii*, the most important ones are anthelmintic, anticancer, anti-



inflammatory, antioxidant, aphrodisiac, diuretic and laxative. Leaves and seed paste are used to treat burning sensation, filarial, inflammatory and eye diseases (Samal and Dehury 2016). Seed paste had been used in the treatment of various diseases like elephantiasis, constipation, ophthalmic, semen disorders, infertility and diseases of the female genital. The bark and seeds are used as an antidote in the treatment of snake bites. The leaves are used in treating illness, phlegm, skin ailment, aridity, and are also helpful in curing rheumatism. *P. roxburghii* possess antioxidant, antipyretic, and anti-inflammatory activities (Pandey and Flulara 2022). Almost all parts of the plant such as bark, stem, leaves, root, fruits, and seeds contain numerous secondary metabolites such as flavonoids, phenolics, triterpenes, saponins, glycosides, alkaloids, saponins, and glucosinolates. The presence of these phytochemicals imparts efficient protection roles against various diseases. Various plant parts and their extracts can be used as a cure for different diseases, including cancer (Gupta 2016; Kumar *et al.* 2019; Naik *et al.* 2023).

Phytochemical information of plants is required to fully explore the different parts of plants and correlate its activity. *P. roxburghii* has remarkable ethnomedical significance. However, its phytochemical profile in different parts has not been fully explored. In this study, various extracts, including aqueous, methanol and hydro-ethanol were prepared from the seeds of *P. roxburghii* and subjected to phytochemical assessment. The qualitative analysis revealed the presence of all representative groups, except tannins and glycoside, in the samples. The primary objective of this study was to carry out an in-depth screening of the plant's phytochemical composition qualitatively and quantitatively.

## MATERIALS AND METHODS

### *Collection of plant samples and identification*

The seeds of plant were collected from University of Agriculture, Faisalabad (UAF) and authentication of plant was done by a taxonomist at the department of Botany at UAF. The photographic documentation of plant and a voucher sample was provided under reference number (255-1-2023) and identified as *P. roxburghii* (Fig. 1).

### *Preparation of seed extract*

The seeds of *P. roxburghii* were washed and subjected to drying under shade. The dried seeds were subsequently ground using a high-speed blender and preserved in an airtight jar. *P. roxburghii* seed powder was used for preparing various extracts. For each extraction, 10 g of the powder was placed in 250 mL conical flasks, and 100 mL of methanol, hydro-ethanol (a mixture of water and ethanol in a 1:1 ratio) and distilled water were added separately. The extraction process was done by using an orbital shaker (IRMECO) set

at 220 rpm for 24 h. Following the extraction period, the resultant mixture was filtered using Whatman No. 1 filter paper. The filtrates obtained were subjected to evaporation at 45°C using a vacuum drying oven (Memmert, GmbH, Dusseldorf, Germany) to obtain the dry extract. Subsequently, these dry extracts were transferred to Eppendorf's and preserved at 4°C for subsequent use. For analysis, evaporated extracts weighing 50 mg were dissolved in 5 mL of DMSO, stored at 4°C and subjected to phytochemical screening and analysis including total phenolic contents (TPC), total flavonoid contents (TFC), and DDPH radical scavenging assay.

### *Phytochemical screening*

The various extracts of *P. roxburghii* seeds were used for qualitative analysis of various groups of compounds, such as flavonoids, carbohydrates, phenols, saponins, alkaloids, glycosides and tannins by employing standard methodologies as presented (Sharma 1995; Treare and Evans 1985; Peach and Tracy 1956; Varma *et al.* 2010).

**Test for phenols:** Potassium dichromate test: two mL of extract was treated with 5% potassium dichromate solution. Positive result was confirmed by a formation of brown precipitate (for phenol). Ferric chloride test: 2 mL of extract was treated 2–3 drops of 5% ferric chloride solution. Formation of bluish-black color showed presence of phenols and black color showed tannins.

**Test for flavonoids:** Lead acetate test: one mL extract was treated with 1 mL 10% lead acetate ( $\text{Pb}(\text{OAc})_4$ ) solution. Formation of yellow color precipitate indicated the presence of flavonoids.

**Test for tannins:** Braymer's test: 2 mL of extract was treated with 2 mL  $\text{H}_2\text{O}$  and followed with 2–3 drops of  $\text{FeCl}_3$  (5%). Green precipitate proved the presence of tannins.

**Test for saponins:** Foam test: two mL extract was diluted with 10 mL of distilled water and warmed gently. It was shaken for 5 min. Persistent froth indicated the presence of saponins. The same extract was added with a few drops of olive oil. Formation of a soluble emulsion confirmed the presence of saponins (Treare and Evans 1985).

**Test for glycosides:** For Keller Kiliani test of glycosides, 2 mL extract was treated with 1 mL glacial acetic acid, one drop of 5%  $\text{FeCl}_3$  and 1 mL of conc.  $\text{H}_2\text{SO}_4$ . The brown ring of the interface indicated the presence of cardiac glycosides (Treare and Evans 1985).

**Test for alkaloids:** Wagner's test: Two mL of extract was treated with few drops Wager's reagent. Formation of reddish-brown precipitate indicated the presence of alkaloids (Treare and Evans 1985).

**Test for sterols:** Salkowski's test: two mL of extract was treated with 2 mL chloroform and 2 mL of conc.  $\text{H}_2\text{SO}_4$ . Chloroform layer appeared red, and the acid layer showed greenish yellow fluorescence, which indicated the presence of sterols.



**Fig 1:** Leaves (left) and fruits (right) of *P. roxburghii*

**Test for terpenoids:** Salkowski's test: Two mL of chloroform and 1 mL of conc.  $\text{H}_2\text{SO}_4$  was added to 1 mL of extract and observed for reddish brown color that indicated the presence of terpenoids (Sharma 1995).

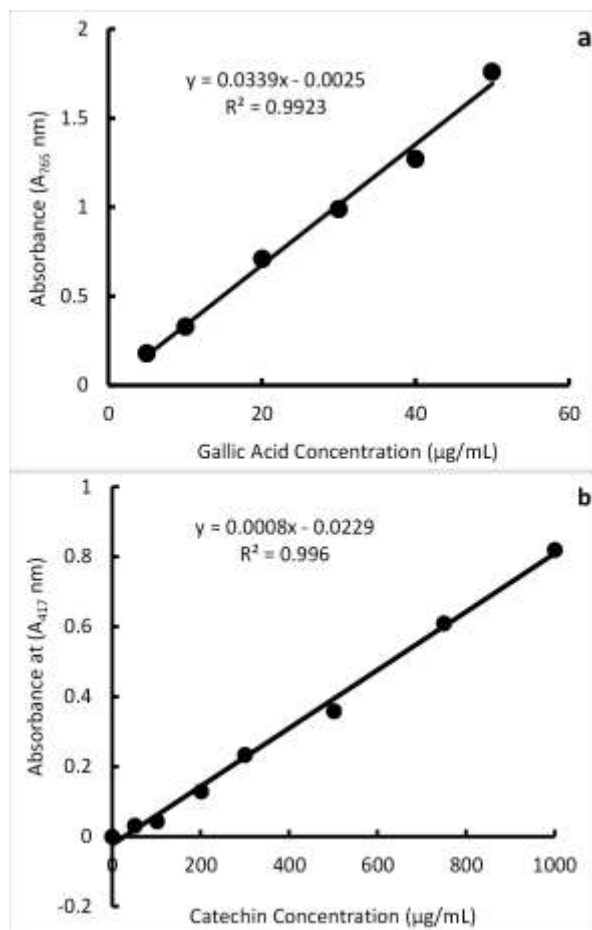
**Test for coumarins:** Two mL of extract was treated with 3 mL of 10% NaOH solution. Yellow coloration indicated the presence of coumarins.

**Analysis of Total phenolic and flavonoids contents:** A quantitative assessment was conducted to determine Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC) in the raw extracts was carried out by following standard protocols.

**Total phenolic content:** The total phenolic contents in the extracts were determined by using Folin-Ciocalteu procedure with Folin-Ciocalteu reagent (MERCK). Standard concentrations of 5, 10, 20, 30, 40, and 50  $\mu\text{g/mL}$  of Gallic acid were prepared from a stock solution of Gallic acid (1 mg/mL) in methanol. For each standard, 100  $\mu\text{L}$  was combined with Folin-Ciocalteu reagent, vortexed, and then 800  $\mu\text{L}$  of sodium carbonate was added and vortexed. After 1 h incubation, the absorbance was recorded at 765 nm with a spectrophotometer using a blank to set the instrument at zero. The blank was composed of 100  $\mu\text{L}$  of methanol instead of the standard. The determination of total phenolic contents (TPC) was conducted for crude extracts obtained from methanol, hydro-ethanol, and water were determined following the same procedure as the standards (Jagadish *et al.* 2009). The findings are presented in equivalence to Gallic acid (mg GAE/g). The standard curve of gallic acid with the equation are given in Fig. 2a.

#### Total flavonoid contents

The determination of Total flavonoid content was conducted using the aluminium chloride method. A range of standard concentrations (50, 100, 200, 300, 500, and 1000  $\mu\text{g/mL}$ ) of Catechin was prepared from a stock solution of Catechin (1 mg/mL) in methanol. To 1 mL of each standard, 1 mL of 2%  $\text{AlCl}_3$  was added, vortexed, and absorbance was measured by using spectrophotometer at 417 nm after a 15 min incubation. The spectrophotometer was set at zero by using blank and it was prepared by substituting 100  $\mu\text{L}$  of methanol in place of standard. The total flavonoid contents (TFC) of crude extracts from methanol, hydro-ethanol, and water were determined



**Fig. 2:** Standard curve of gallic acid (a) and catechin (b)

following the same procedure as the standards (Riaz *et al.* 2019). The results are expressed in equivalence to catechin (mg Catechin E/10 g). The standard curve of catechin with the equation are presented in Fig. 2b.

#### DPPH radical scavenging assay

The antioxidant capacity of the extracts was determined by their capability to neutralize DPPH assay (Riaz *et al.* 2019). In a 250  $\mu\text{L}$  plant extract, 1 mL of the DPPH solution was added, thoroughly mixed, and the mixture was left for 30 min for incubation in darkness. Subsequently, the spectrophotometer was used to measure the absorbance of both DPPH and extracts at 515 nm. The spectrophotometer was set at zero with blank (methanol) (Gulcin and Alwasel 2023). The percentage DPPH scavenging capability was calculated using the formula given below:

$$\% \text{ inhibition} = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

Where  $A_{\text{control}}$  is absorbance by the DPPH and  $A_{\text{sample}}$  is absorbance by the test samples

### Statistical analysis

Triplicate analysis was done for each parameter and the data have been presented as mean  $\pm$  standard deviation of triplicate analysis. Correlation coefficient ( $r$ ) of phytochemical activities was calculated by employing the correlation and regression function of Microsoft Excel program (Microsoft, Redmond, WA, USA).

## RESULTS AND DISCUSSION

### Importance of phytochemical constituents

Phytoconstituents are generated by the plants as a defense system against pathogens and predators. They are helpful for the treatment of diseases including antimicrobial, antioxidant, and stimulation as well as inhibition enzymes (Ahmed *et al.* 2017). Plant-based therapeutic agents possess fewer side effects as compared to synthetic agents and are also cost-effective (Mustafa *et al.* 2017). Plants contain a wide range of constituents such as alkaloids, polyphenolics, tannins terpenoids, etc. which are attributable to therapeutic potential (Verma and Singh 2008). Phytochemicals, either as crude extracts or isolated compounds, provide opportunities for drug discovery (Sasidharan *et al.* 2011). The various extracts of seeds were used for qualitative analysis of various groups of compounds, such as flavonoids, carbohydrates, phenols, saponins, alkaloids, glycosides and tannins by employing standard methodologies as presented in Table 1 (Auwal *et al.* 2014; Gul *et al.* 2017; Amine *et al.* 2019; Hussain *et al.* 2023). Furthermore, it aimed to determine the qualitative phytochemical composition and antioxidant potential as TPC, TFC and DPPH % qualitatively of seed extracts of *P. roxburghii*.

### Qualitative phytochemical screening

A comprehensive estimation of phytochemicals within their respective categories was carried out in *P. roxburghii*. Positive and negative results were obtained for all tests conducted. According to the identification of noteworthy chemicals entitles the various extracts of *P. roxburghii* seeds, including methanol (M), Hydro ethanol (HE) and aqueous (A) extract. The results of qualitative screening of phytochemicals of *P. roxburghii* seeds showed the presence of Phenols, Flavonoids, Saponins, Alkaloids, Carbohydrates, Sterols and Terpenoids in all examined samples (Table 1). Our results showed absence of tannins and glycosides in all extracts which are in line with Siwach *et al.* (2024) but contrary to Sarath and Sudha (2019) that shows presence of tannins in methanolic extract. Siwach *et al.* (2024) reported absence of cardiac glycosides, tannins and saponins in methanolic extracts of seeds of *P. roxburghii*.

Different parts like leaves, fruits, seeds, root and stem bark of *P. roxburghii* showed the presence of many phenols, alkaloids, saponins, steroids, flavonoids and glycosides and triterpenes (Raghavendra *et al.* 2010; Kumar 2020; Balkrishna *et al.* 2021; Mishra *et al.* 2023). Qualitative phytochemical tests help to understand the role of chemical compounds and their usefulness as pharmaceuticals (Gupta 2016; Emasushan and Jhon Britto; 2018; Pandey and Fulara 2022).

### Total phenolic content

The results of quantification of total contents of phenols and flavonoid in the various extracts of the *P. roxburghii* seeds are presented in Table 2. The determination of TPC is expressed as milligrams of Gallic acid equivalent per grams of the extract, determined using a reference standard curve (Fig. 2). The contents exhibited variation among fractions. Total phenolic content ranged from  $48.8 \pm 3.29$  to  $83.0 \pm 2.88$  mg GAE/g of extract. The hydro-ethanol extract exhibited the highest phenolic contents ( $83.0 \pm 2.88$  mg/g of GAE), followed by the methanol extract with phenolic quantities of  $65.0 \pm 9.5$  mg/g of GAE. In comparison the aqueous extract displayed a lower phenolic concentration ( $48.8 \pm 3.29$  mg GAE/ g). These results align with findings reported in leaf by Keshav *et al.* (2021) reported TPC in hydro ethanol (30:70) leaf extract of *P. roxburghii* as  $46.58 \pm 2.52$  mg GAE/g which is lower than from present study. Shahwar *et al.* 2012 reported total phenols as  $176.0 \pm 1.3$  and  $36.9 \pm 3.0$  mg GAE/g of stem extract of methanol and distilled water, respectively. These values are higher for methanol extract and lower for water extract. TPC notified in our research was somewhat higher than a recently published study which revealed that *P. roxburghii* leaf (hydroethanol, 30:70) extract contains  $46.58 \pm 2.52$  mg/g GAE polyphenolic content (Keshav *et al.* 2021). Alterations in agro-climatic conditions accompanied with temperature and rainfall impart a significant impact on the amount of phytoconstituents within similar species of plants growing in different regions (Kumar *et al.* 2017) and different parts of the same plant also show variation in TPC and TFC contents (Shahwar *et al.* 2012; Sarath and Sudha 2019; Nazli *et al.* 2022). Hence, differences in solvent composition, plant collection sites and parts might be responsible for the variations of estimated TPC.

### Total flavonoid content (TFC)

The results of total flavonoid contents as in the extracts are presented as milligrams of catechin equivalent per 10 g of the extract are presented in Table 2. The flavonoid quantities ranged from  $565 \pm 121$  to  $915 \pm 185$  mg CE/10 g. Hydro-ethanol extract exhibited the highest flavonoid content ( $915 \pm 185$  mg CE/100 g), followed by the aqueous extract ( $728 \pm 130$  mg CE/10 g) while the methanol extract displayed the least quantity ( $565 \pm 121$  mg CE/10 g). The observed flavonoid contents in *P. roxburghii* seeds extracts were higher than previous reported (Nazli *et al.* 2022; Keshav *et al.* 2021).

**Table 1:** Qualitative analysis of phytochemicals in extracts derived from *P. roxburghii* seeds

Extracts	Tests	Methanol	Hydro-ethanol	Aqueous
Phenols	Potassium dichromate test	+	+	+
Flavonoids	Lead acetate test	+	+	+
Tannins	Ferric chloride test	-	-	-
Saponins	Foam test	+	+	+
Glycoside	Keller Kiliani test	-	-	-
Alkaloids	Wagner's test	+	+	+
Carbohydrates	Molisch test	+	+	+
Sterols	Salkowski's test	+	+	+
Terpenoids	Salkowski's test	+	+	-
Coumarins	10 % NaOH test	+	+	+

Methanol (M), hydro-ethanol (HE) and aqueous (A). (+) indicates the presence of observed representative groups. (-) indicates the absence of observed representative groups.

**Table 2:** Total phenolic and flavonoid contents, and DPPH percent inhibition of seeds of *P. roxburghii* in different extracts

Extracts	TPC (mg/g) <sup>i</sup> (GAE)	TFC (mg/10g) <sup>ii</sup> (CE)	DPPH (% inhibition)
Methanol	65.0 ± 9.5	565 ± 121	69.2 ± 8.0
Hydro ethanol	83.0 ± 2.88	915 ± 185	32.8 ± 1.86
Aqueous	48.8 ± 3.29	728 ± 130	35.6 ± 9.60

Methanol (M), hydro-ethanol (HE) and aqueous (A) (i) Total Phenolic Contents (TPC) were quantified in milligrams of gallic acid equivalents (GAE) per grams of extract. (ii) Total Flavonoid Contents (TFC) were measured in mg of catechin equivalents (CE) per 10 g of extract. Values are presented as means ± standard deviation (SD). Ascorbic acid served as the positive control (n=3).

#### DPPH radical scavenging assay

Metabolic processes within the body and environmental factors produce free radicals mainly reactive oxygen species (ROS) which cause various ailments including ageing, carcinogenesis, mutagenesis, and cardiovascular abnormalities. Antioxidants are the agents which counteract the effects of free radicals and limit oxidative stress (Kedare and Singh 2011). DPPH assay is a standard method to investigate the free radical scavenging ability of test samples (Mishra *et al.* 2012). The ability of various solvent extracts to donate hydrogen atoms or electrons was assessed by reducing a purple DPPH into 1,1-diphenyl-2-picryl hydrazine (Raclariu-Manolică and Socaciu 2023). The ability of each extract to scavenge DPPH radicals was then measured and presented in Table 2. The methanol extract demonstrated DPPH radical scavenging activity  $69.2 \pm 8.0\%$  whereas hydro ethanol and aqueous extract show almost similar activity as  $32.8 \pm 1.86$  and  $35.6 \pm 9.60$ , respectively. The plants rich in phenols and flavonoids possess substantial antioxidant potential and are believed to be accountable for the observed antioxidant capacity in the DPPH experiment. Nazli *et al.* (2022) reported maximum percent free radical scavenging activity by methanol stem (MeOH-S) and distilled water leaf (DW-L)-L extracts as  $86 \pm 0.56\%$  which is higher than our study.

#### CONCLUSIONS

Based on the results, it has been established that hydro ethanolic extract of *P. roxburghii* seeds contain high contents of both TPC and TFC while methanolic extract showed highest percentage of DPPH inhibition. *P. roxburghii* seeds can be used for free radical scavenging and attributed to its flavonoids and phenolic acids. However, further testing is

necessary to demonstrate specific chemical composition.

#### AUTHOR CONTRIBUTIONS

MJ did analytical work; TI conceptualized and supervised the work; SZ and RR wrote and reviewed the first draft; MS performed formal data analysis, partially supervised lab work. All authors read and approved the final draft.

#### CONFLICTS OF INTEREST

The authors affirm that they possess no conflicts of interest.

#### DATA AVAILABILITY

The data will be made available on a fair request to the corresponding author

#### ETHICS APPROVAL

Not applicable

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## A Review of the Teratogenic Effects of Drugs and Environmental Toxins

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### ABSTRACT

**Background:** Teratogenesis is the formation of an abnormal or deformed body. It is a process of inducing birth defects in fetus. Birth defects include brain injury, heart abnormalities, kidney defects or defects in spinal cord. It occurs if teratogenic agents (chemical agents, metal, pharmacological agents or environmental toxins) being exposed to fetus during an organogenesis period of 3–8 weeks. Presently the prevalence of teratogenic effect varies according to specific drug, genetic basis, population etc.

**Objective:** The purpose of this review is to understand the mechanism behind the teratogenic effects, risk factors and to control their exposure to pregnant women.

**Methodology:** The information for this article was collected from different sources including Elsevier, Springer, Taylor & Francis, Google Scholar, Web of Science, Scopus, HEC Digital Library and other online sources.

**Results:** Various drugs have been reported to cause teratogenicity to the fetus. These include thalidomide, isotretinoin, phenytoin and valproic acid, alcohol etc., which adversely affect fetus organ development and tissue formation by impeding cell division, which is necessary for blastocyte formation and organ development. Moreover, environmental toxins including heavy metals (e.g., mercury, lead, and cadmium) act as carcinogen, immunotoxicant, cause malformations and even death in embryos and fetuses. The mechanism behind the teratogenic effects includes folate antagonism, endocrine disturbance, oxidative pressure, vascular disturbance and specific receptor and enzyme mediated teratogenesis. The factors that increase the risk for teratogenic effects include poor maternal nutrition, maternal age (>35) and maternal health conditions. It also includes discontinuation of medications related to acute or chronic conditions.

**Conclusion:** Preventive measures should be taken to avoid teratogenic effects as the period of the first trimester is most important for the organ development of fetus. Hence, it is necessary to avoid the exposure of teratogens. Taking herbal therapy, neuropathic therapy and acupuncture therapy play a great role in promoting good maternal health and prevent adverse teratogenic effects.

## INTRODUCTION

Teratogenicity, also known as reproductive and developmental toxicity, has emerged as a vital component of toxicology overall. Each substance possesses a distinct toxicological profile and a specific mechanism of teratogenicity (Dron 2016; Melo *et al.* 2021). The investigation of teratogenicity is crucial for comprehending the potential dangers linked to various substances and agents

(Valladares and Rasmussen 2022). This understanding is essential for averting birth defects and promoting the health and welfare of future generations (Ananya 2016).

While the use of prescription medications is prevalent during pregnancy, the teratogenic risks to humans remain unclear for over 90% of drug treatments that have been authorized in the USA in recent decades. A specific birth defect can originate from various mechanisms and multiple encounters, including those from medicines. A particular





pathogenic mechanism may lead to different results influenced by factors like the embryonic age at exposure, the length of time and amount of encounter, and inherited predisposition (Tona *et al.* 2022). Teratology has operated as a descriptive science centered on identifying birth defects via clinical or laboratory techniques (Lupo *et al.* 2024). Commonly used animal testing systems have demonstrated varying degrees of success in recognizing chemical teratogens.

With the rise of epigenetic research concerning developmental processes, it is now feasible to prevent certain birth and developmental abnormalities through dietary and behavioral modifications. Nonetheless, the prevention of birth defects stemming from genetic origins through gene editing has become a reality, provoking ethical dilemmas (Stump *et al.* 2012).

## PROCESSES OF TERATOGENICITY

Biochemical investigations hold an essential role in the comprehensive strategy for exploring human birth defects. Nevertheless, well-integrated biochemical tests can enhance and question the outcomes from studies reliant on morphological and ultra structural methods (Gomes *et al.* 2021). The theoretical framework of this field remains limited to just a handful of 'rules of thumb' hypotheses. Additional research is necessary to comprehend the biochemical processes that drive teratogenicity. Radiation teratogenesis is an intricate phenomenon. Before reaching the blastocyst phase, the embryo shows no sensitivity to the teratogenic and growth-inhibiting effects of radiation (Applegate *et al.* 2021). In the initial stages of organogenesis, the embryo becomes susceptible to the growth-inhibiting, teratogenic, and fatal consequences of irradiation. The influence of radiation on the developing embryo is contingent upon both the developmental stage and the radiation dose. There may be shared mechanisms between carcinogenesis and teratogenesis. The intricate mechanisms behind carcinogenesis and teratogenicity remain largely unclear. Nonetheless, some recognized or potential common mechanisms include mutations in genes or chromosomes, disruptions in gene expression, modified membrane characteristics, or changes in intracellular balance.

Teratogens are agents that could lead to physical or functional malformations in a human embryo or fetus following the encounter of the pregnant woman to the agent (Paredes-Páliz *et al.* 2024). Alcohol and cocaine serve as examples of such agents. The effect of the teratogen on the fetus or embryo varies based on factors such as exposure duration, quantity of teratogenic agent, and developmental stage (Mohammadi 2022; Mainprize *et al.* 2023). Vitamin A is an essential dietary nutrient. However, excessive intake of retinoids by expectant mothers can lead to teratogenic outcomes (Saurat and Sorg 2023). The incidence and nature of fetal malformations linked to maternal consumption of both natural and synthetic retinoids have been thoroughly

documented. Major congenital malformations are a key contributor to infant illness and death and represent a significant societal and financial burden. Depleted uranium has been associated with teratogenicity, signifying the potential of a substance to induce birth defects. Epidemiological research has examined the possible health impacts of exposure to depleted uranium (Ran *et al.* 2020). Now the agent is recognized as a teratogen if its administration to the pregnant woman leads, either directly or indirectly, to systemic or practical abnormalities in the fertilized conceptus, and trigger abortion of the early embryo, late fetal mortality, congenital anomalies, or inside the uterus growth restriction (Friedmacher and Jesudason 2023).

## MECHANISM OF TERATOGENICITY

Teratogenicity involves the stimulation of the transcription of  $\alpha$ v and  $\beta$ 3 integrin subunit genes by insulin like growth factor I (IGF-I) and fibroblast growth factor 2 (FGF-2) (Fig. 1). The resulting  $\alpha$ v- $\beta$ 3 integrin dimer promotes formation of new blood vessels in the developing limb bud, facilitating the outgrowth of the sprout (Tsamantioti and Hashmi 2024). Isotretinoin is a highly effective treatment for acne that works by inducing apoptosis in sebocytes. However, it also has teratogenic effects, leading to birth defects due to its influence on neural crest cells. Furthermore, isotretinoin can cause depression, mucocutaneous side effects, loss of hair, inflammatory bowel disease, muscle pain, and damage to the liver (Draghici *et al.* 2021). The underlying mechanism for these adverse effects is apoptosis, which could be affected by genetic variants (Melnik, 2017). Anticonvulsant medications, utilized to treat epilepsy, also carry teratogenic hazards. Pregnant women affected by epilepsy are at an elevated risk of congenital malformations, miscarriages, and stillbirths (Draghici *et al.* 2021). A 30-year review of medical literature substantiates this heightened risk. Therefore, careful management and consultation with healthcare professionals are crucial for pregnant women with epilepsy.

## TERATOGENIC DEFECTS DUE TO DRUGS AND HEAVY METALS

### *Teratogenic medicinal agents*

Some of the possible and reported birth related defects due to medicinal agents are basically the defects relevant to brain injury along with, malformations in the spinal cord, congenital abnormalities, renal fault, GIT problems and all the problems associated with imperforate anus (Shernazarovna, 2024). Many medicinal agents are known to cause congenital defects or birth defects some of them are sodium valproate, alcohol, isotretinoin, aminopterin, cocaine, warfarin, thalidomide, tetracycline, coumarin, buprenorphine, etretinate and phenytoin (Abadie *et al.* 2023), as discussed below.



**Fig. 1:** Mechanism of teratogenicity

Drugs use in pregnancy at any time during pregnancy can affect the fetus and cause numerous diseases in fetus and embryo. If the pregnant women suffering from acute or chronic condition and take medication so discontinue the medication which is against the chronic condition so, it prevents the fetus from abnormalities. Does not use the over-the-counter drugs during pregnancy take multivitamins and folic acid during pregnancy because these prevent the adverse pregnancy outcome. For the pregnant women medication always chooses from pool of effective drugs so that prevent the congenital development disorders (El Shamy and Tamizian 2018). Avoid the use of Drugs like anti- epileptic, ACE inhibitors, Antibiotic, thalidomide, and NSAIDs cause the structural and functional defects in fetus. For the protection of fetus use effective strategies like patient education and use safer therapeutic alternatives (Sun *et al.* 2022). Provide the patient counselling about the medication which are harmful during pregnancy. Develop and implement the risk management program so effective outcome achieve (Griffin *et al.* 2018; Khadivzadeh *et al.* 2023).

**Isotretinoin:** The intake of isotretinoin by pregnant lady increases the risk of teratogenic malformations or deformities by 25% (Brzezinski *et al.* 2022). Buildup of CSF in brain abnormally a condition also known as hydrocephalus, microcephaly, a structural defect in head of ana infant, cerebellar hypoplasia, depressed nasal bridge, microtia or absence of outer ears, cleft palate, aortic arch defects, heart anomalies (ventricular septal defect, atrial septal defect, tetralogy of Fallot), and hypoplastic adrenal cortex included in the defects. Furthermore, there is an increase in unplanned abortions. Isotretinoin (analogue of retinoic acid) is a dermatologically active compound reported to cause malformations in fetus when mother is on the intake of it. In first trimester if the pregnancy its oral use is strictly prohibited (Altıntaş Aykan and Ergün 2020).

**Etretinate:** Etretinate and its active metabolite acitretin can cause teratogenicity. Parturiency should not be planned in the course of the treatment and even after 2 years of treatment termination (Jeong *et al.* 2022). The most important and functional knowledge is given by reports in females who

were vulnerable to either retinoid before or during pregnancy (Menezes and Almeida 2024). This information shows an increased risk of spontaneous abortion or congenital mutation when the drug is taken during the first trimester of gestation (Jeong *et al.* 2022). After treatment has stopped, the risk is considered to be low since the number of problems seems not to surpass those noticed in a widespread inhabitant. Teratogenicity is the possible side effect of oral retinoid therapy and the risk with etretinate is particularly high because of its ability to store in fat and continues release after discontinuing the treatment embryonic malformation was observed in a pregnancy occurred almost a year after the last dose of the drug (Jeong *et al.* 2022). Moreover, Etretinate can result in skeletal, cardiovascular, and central nervous system malformations, just like its congener isotretinoin. Etretinate, in contrast to isotretinoin, tied to lipoproteins and remain longer in the bloodstream even for years after treatment (Sarkar and Meena 2023).

**Thalidomide:** The crucial phase was not more than 14 days after gestation, and the delicate period for the occurrence of human thalidomide birth defects was 23 to 28 days post conception (Sarkar and Meena, 2023). Throughout this time, before birth encounters give rise to about 20% of births to result in newborns with defects, the most usual of which were limb deformities ranging from tetra-amelia or phocomelia of the upper and lower limbs to triphalangeal thumb, seldomly with preaxial polydactyl of six or seven toes of each foot. In over 10,000 children, thalidomide caused severe birth defects between 1957 and 1962. Any tissue or organ of fetus could be affected by thalidomide. Due to the extensive mangle and circumstances that thalidomide exposure can result in, the damage is commonly referred to as thalidomide embryopathy or thalidomide syndrome (Vargesson 2019). Damage to limbs is one of the most ordinary features. phocomelia remains the most obvious limb defect produced by thalidomide. The legs can also reveal thalidomide-induced problems. Phocomelia or Amelia are seen, as well as shortening in the long bone. Nevertheless, the malformations of the lower limbs are seen infrequently as those of the upper limbs. Damage to the face is another key symbol of thalidomide embryopathy. At birth the existence of hemangioma, that is also known as a “storkmark”. The facial asymmetry and facial palsy because of the feeble facial muscles and facial nerve damage is a hallmark of thalidomide teratogenicity (Yamanaka *et al.* 2021). Cases report that about 20% birth anomalies was caused by prenatal exposure to it. The frequent of which were limb deformities including phocomelia of the upper and lower limbs to triphalangeal thumb, sometimes accompanied by thumb duplication also known as preaxial polydactyly in which a baby has six or seven toes each foot (Anonymous 2018). Moreover it may cause anomalies of limbs, urinary tract, facial weak matches, esophageal and duodenal atresia, cardiac defects and a condition in which kidneys fail to develop (Vianna *et al.* 2017).

**Ergotamine:** Ergotamine is an alkaloid of ergot present naturally that has ability to contract smooth muscles. Females

who used ergotamine for the cure of migraine in the first trimester of their pregnancies gave birth to babies with neural tube defects (Bérard *et al.* 2021). Ergot toxicity which can result in acute vasospasm does not spare the fetus or mother so it should not be considered without risk. The correlation between low birth weight or premature birth & ergotamine medical care could be attached with ergotamine produced narrowing of vessels in the placenta of expecting women (Aukes *et al.* 2019; Bérard *et al.* 2021). Genitourinary malformations in relation to ergotamine usage were reported rarely but 20% cases of renal agenesis had been reported.

**Trimethadione:** About 1/4<sup>th</sup> of pregnancies results in immediate abortion due to maternal utilization of Trimethadione. Most of infants have deformities, developmental slow up, and prenatal and postnatal growth insufficiency (Andrews *et al.* 2019). These deformities include brachycephaly with mid facial hypoplasia, broad nasal bridge, V-shaped eyebrows with or without synophrys, arched or cleft palates, and misplaced ears. Tetralogy off a lot and septal defects are common cardiovascular problems. Other frequent conditions include kidney deformities, trachea esophageal abnormalities, hernias, and hypospadias. Mental retardation and mild to moderate speech difficulties has been observed I survive (Tarate *et al.* 2022). During early embryonic stage the administration of drug over the phase of first trimester causes early embryonic organogenesis and caused 80% embryo mortality. Apart from that the very frequent defects involve cleft palate, cardiac defects, urogenital deformities, and skeletal problems. Slow mental and physical growth was observed as well. However, anticonvulsant Trimethadione is a highly potent inducer of ventricular septation defects and many other congenital defects so its use in pregnancy is highly discouraged (Kaleelullah and Garugula 2021).

**Phenytoin:** Phenytoin is an anticonvulsant medication that is utilized for curing epilepsy. If consumed by the mother in the first trimester, there are chances for a series of congenital defects known as the fetal hydantoin syndrome. The pattern of abnormalities consists of developmental detain or frank mental inadequacy, dysmorphic craniofacial traits, and hypoplasia of the distal phalanges (Tsamantioti and Hashmi 2024). The existence of vital phenytoin-associated birth defects in little ones relates with an incapability of lymphocytes to remove the drug. There appears to be inheritable vulnerability to phenytoin fetal defect. The chance of growth delays in phenytoin encountered children ranges from 1 percent to 11 percent. Moreover benzodiazepines could cause apnea, hypotonia, hypothermia, and neonatal abstinence syndrome with signs and symptoms of neuromuscular excitability (Ritchie *et al.* 2021). Additionally, if at all possible, it should be discontinued at least two weeks before conception (Cassina *et al.* 2012). The incidence of anomalies, primarily cleft palate, and decrease in maternal plasma folate levels on day 12 of pregnancy. Also it has the ability to bring about embryonic hypoxia mediated

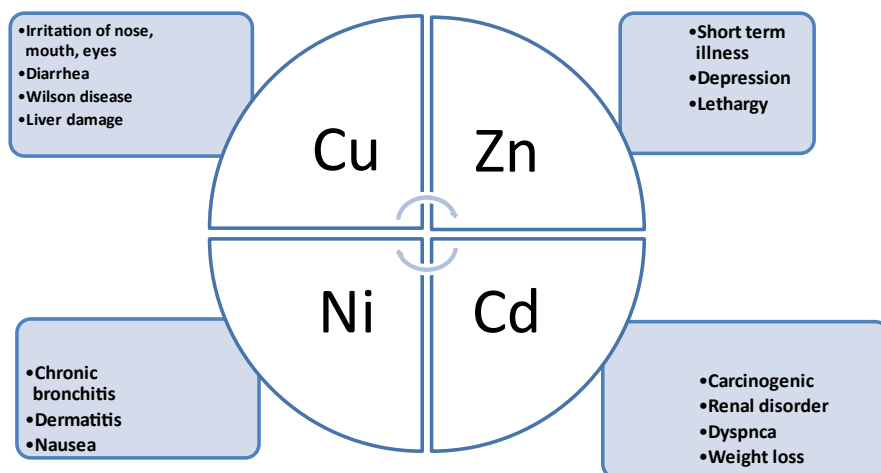
via bad effects on the heart of embryo (Marefat and Sadeghi 2020).

#### *Environmental toxins and their effects*

Heavy metals are naturally found elements that increased density and can be poisonous even in small amounts. These elements have elevated atomic weight and are at least five times denser than water. Examples include lead, arsenic, mercury, cadmium, silver, copper, iron, chromium and nickel (Cooper *et al.* 1984). These metals can be found in the surroundings naturally, or as a result of industrial countries with more industrial development. Metals such as lead, silver, cadmium and mercury, along with metalloids such as arsenic, are causative agents of reproductive toxicity. The metals released by industries can damage the soil, and because these metals accumulate through the food chain, they eventually pose a significant risk to human health. These metals are found in higher concentrations in plants and aquatic animals than what is considered safe by the World Health Organization (Ashraf *et al.* 2019). Environmental degradation also increases the risk of human exposure to heavy metals, leading to health issues like reproductive problems.

These metals affect female reproduction at every stage, from development to maturation and hormone regulation, and are linked to higher rates of infertility in women (Fig. 2). Long-term exposure may result in serious conditions such as menstrual issues, miscarriages, preterm births, and stillbirths (Agarwal *et al.* 2012). Heavy metals can alter neurotransmitter activity in the CNS and disrupt the normal release of gonadotropin-releasing hormone (GnRH) from the hypothalamus (Gerhard *et al.* 1998). High levels of these toxins in infants are linked to issues such as low birth weight, developmental and behavioral problems, as well as growth restrictions and delays during fetal development (Palmieri *et al.* 2019). One of the factors studied for decreased birth weight is exposure to heavy metals, including cadmium, mercury, lead, arsenic and zinc. These metals are particularly concerning for maternal reproductive health and fetal wellbeing because they can cross the placenta, leading to potential toxicity in the developing fetus (Sabra *et al.* 2017). Three most noxious teratogenicity causing heavy metals are described below.

**Mercury (Hg):** Hg contamination can happen through food, water, air, or skin contact. It may be the only thing that builds up in the food chain and can effortlessly change into more poisonous organic forms, like methyl mercury (MeHg), dimethyl mercury, and ethyl mercury. The effects of Hg exposure vary widely, depending on factors like the level and duration of exposure, as well as the age and health of the individual (Zulaikhah *et al.* 2020). Hg contamination in the environment is primarily caused by human activities, such as coal burning, mining, cement production, and the chemical industry. Once Hg is released into the environment,



**Fig. 2:** Heavy metals causing potential toxicity in fetus

groundwater, bacteria and other microorganisms often play a key role in transforming it into MeHg, a more toxic form of Hg. Organic types of Hg are more harmful than their inorganic counterparts (Bjørklund *et al.* 2019). MeHg is fat-soluble, which means it can easily cross the cell membranes. It can pass through the placenta at a rate 10 times higher than other Hg compounds (Dutta 2015). Organic Hg also easily passes into breast milk so the infants who are breastfed are more vulnerable to Hg toxicity. Infants exposed to methylmercury can experience damage to the nervous and immune systems, DNA repair disruptions, destruction of mitochondrial membranes and neurodegenerative diseases.

**Cadmium (Cd):** It is heavy metal present naturally in our environment. It enters into the human body at workplaces, through nutritional meals, or utilizing contaminated water. It is capable to disrupt body hormonal balance so it disrupts endocrine level (Ali *et al.* 2023). Moreover it alters the natural steroid synthesis process, cause miscarriage, interferes the menstrual cycle and cause birth defects in neonates (Ali *et al.* 2023). It is also examined that Cd interferes with methylation of DNA that affects both mother and fetus (Moynihan *et al.* 2017). It taking notice of action ways of cd includes the mechanism of oxidative stress that induces cell death and cause irreversible harm to mitochondria and endoplasmic reticulum organelles of cell (Jacobo-Estrada *et al.* 2017). Women are more likely to affect by cd instead of men. Cd-induced birth defects include clubfoot, spinal cord defects, lung abnormalities, fluid accumulation in brain.

**Lead (Pb):** In developed and developing countries, Pb toxicity is becoming a major health problem. It cause immune imbalance, infertility, vitamin D deficiency (Mitra *et al.* 2017). Exposure to this metal causes developmental problems including premature birth and pregnancy loss. When Pb amount increases up to a highly toxic level, it causes abortion. It effects DNA transcription cause damage to cell structure and cell membrane (Collin *et al.* 2022). Along with calcium greater amount of Pb passes from

maternal to fetus through placenta and after birth it transfer to baby through breast milk (Yurdakök 2015). In fetus calcium transfer from mother is maintained but 1,25 dihydroxy vitamin D. In last trimester which is the period of development of neuronal structures, brain growth, shoulders heel and synapse formation so calcium transfer increase and as Pb follows calcium to pass to fetus through placenta so Pb quantity increase and cause more birth defects (Rísova 2019).

## PREVENTION OF TERATOGENESIS

### *Avoidance from environmental exposure*

Perfluro alkylating Agents are present in industrial and consumer products, which can cause the effect on the immune system and neural defect and fetus growth during pregnancy. Avoidance from the Cd exposure in the course of pregnancy is important because it can result in premature birth and low birth weight. There is a need to minimize exposure to pesticides or organophosphate during pregnancy because it can cause the effect on neural behavior development in fetus. Likewise, exposure to air pollutants during pregnancy can cause developmental disorder in fetus such as impaired lung development, altered immune development and low birth weight (Rani and Dhok 2023). Alcohol consumption during pregnancy leads to fetal suffering from various developmental disorders (Ali *et al.* 2021). Pb exposure during pregnancy be avoided because it causes the abortions and offspring suffering from neuro behavioral development, and reduced fetal growth (Santana *et al.* 2023).

### *Alternative therapy to prevent teratogenesis*

**Nutritional therapy:** Supplements like folic acid or vitamin B9 play an important role during pregnancy, as they are involved in nucleic acid synthesis and it prevents neural tube defects. Chorine is very essential nutrient it protects from

neutral tube defects during pregnancy (Best *et al.* 2022). Omega-3-fatty acid is involved in fetal brain and retina development. Antioxidants such as vitamin C, E, selenium, zinc and manganese in pregnancy prevent the oxidative stress and reduce miscarriage and preeclampsia (Banović Fuentes *et al.* 2024).

**Herbal and traditional therapy:** Herbal and traditional therapy play an important role during pregnancy because it reduces nausea, vomiting other GIT (gastrointestinal tract) related problems and infections. Ginger is used as anti-inflammatory, antioxidant and antihypertensive and it prevent nausea and vomiting during pregnancy (Sarecka-Hujar and Szulc-Musiol 2022). Turmeric reduces oxidative stress and inflammation.

**Naturopathic therapy:** Cholelithiasis of pregnancy is treated with activated charcoal used for detoxification (Smith 2022). Probiotic fermented foods has positive effect on fetal health development (Mauder *et al.* 2024).

**Acupuncture therapy:** It plays a significant role during pregnancy because it promotes the normal birth and reduce stress (Lin *et al.* 2022, da Costa *et al.* 2022).

## CONCLUSION

Selection of appropriate medications and reduction of environmental toxins represent vital pregnancy concerns because teratogenic risks are high. Medical drug avoidance of isotretinoin as well as thalidomide and warfarin are essential while prevention of developmental disorders in the fetus requires managing environmental contaminants such as Pb, Hg and pesticides. The combination of patient education together with risk control measures alongside folic acid and chorine and antioxidant supplementation reduces the potential negative pregnancy outcomes. The combination of herbal medicine and acupuncture therapy provides support to pregnant patients without creating significant birth defects. Healthcare professionals must employ combined medical and environmental and lifestyle strategies to protect fetal wellbeing. The advancement of maternal care research and policy adjustments will lead to better maternal wellness and decreased teratogenic threats.

## AUTHOR CONTRIBUTIONS

Noor Ul Ain: Topic decision in publication, journal approach, publication process, data defining, writing and data collection. Rest of the authors: Data collection, writing of article

## CONFLICTS OF INTEREST

The authors affirm that they possess no conflicts of interest.

## DATA AVAILABILITY

The data will be made available on a fair request to the corresponding author.

## ETHICS APPROVAL

Not applicable

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## Comparative Morphological and Anatomical Studies on Young Leaves of *Ficus* Species

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### ABSTRACT

**Background:** The genus *Ficus* comprises a diverse group of plant species known for their ecological, ornamental, and medicinal value. Its species exhibit significant variation in leaf morphology, anatomical structures, and physiological traits, making them ideal candidates for comparative botanical studies.

**Objective:** A comparative study was conducted to investigate the morphological and anatomical characteristics of selected *Ficus* species collected from the New Botanical Garden, University of Agriculture, Faisalabad, between November and February.

**Methodology:** The second fully emerged leaf from each species was sampled for analysis. Key parameters included leaf area, petiole length, and features of the lower epidermis. Epidermal layers were peeled and preserved in FAA solution for microscopic evaluation of stomatal density, number of subsidiary cells, pore size, and guard cell dimensions.

**Results:** *Ficus elastica* exhibited the highest stomatal density, while *F. natalensis* showed the lowest. The petiole length of *F. elastica* increased gradually over time, whereas *F. natalensis* maintained a consistently short petiole without a clear pattern of change. The leaf area of *F. elastica* decreased from November to December and increased again in January. *F. natalensis* consistently showed the smallest leaf area throughout the study period. The guard cell length of *F. elastica* decreased gradually over time, whereas in *F. hawaii*, it showed an increasing trend. Guard cell width increased in *F. macrophylla* but decreased in *F. elastica* over the study period.

**Conclusion:** Significant anatomical and morphological variations between *Ficus* species were found in this study. *F. natalensis* had the smallest and most stable leaf area with the least amount of variation in petiole length, whereas *F. elastica* displayed the highest stomatal density and increasing petiole length over time. Species-specific responses to seasonal circumstances were recorded in pore diameters, guard cell size, and stomatal features across *Ficus* species and observation months.

### INTRODUCTION

The genus *Ficus* is among the premier genera of shrubs and trees, which are known for its medicinal, socioeconomic and religious importance. As regards its medicinal importance, the *Ficus* spp. are known to biosynthesize bioactive compounds including phenolics, flavonoids, terpenes etc., which are used to treat the ailments such as diabetes, toothache, gum infection, piles etc. (Salehi et al. 2021; Devi et al. 2022). Interestingly all parts of plant including bark,

roots, leaves and fruits are used in the treatment of various ailments (Murugesu et al. 2021).

Morphological features of plants have close association with the biochemical pathways operative in leaves and other parts (Klem et al. 2019). Photosynthesis, which depends upon the stomatal functions in the exchange of water and gases, is one of the fundamental processes involved in the production of primary and secondary metabolites. Hence, the stomatal density and function in the leaves may directly influence the synthesis of primary metabolites and indirectly



the secondary metabolites (Ördög et al. 2013).

Plants have historically been categorised according to the appearance of their fruits and blooms. In order to accurately identify and classify closely related taxa, plant scientists have been using leaf epidermal morphology (Abdulrahman and Oladele 2010; Qiu et al. 2023; Hussain et al. 2025). Moreover, to differentiate between various taxonomic ranks, such as families, tribes, genera, and species, morphological traits are essential (Alaïda and Aldhebiani 2022). However, several species of flowering plants have similar morphological attributes, so it might be difficult to differentiate them based only on morphological traits (Nazir et al. 2013). Determining the taxonomic placements of these species thus requires an awareness of both the similarities and differences within a sub-genus. Numerous crucial micromorphological characteristics were identified through the examination of *Ficus* epidermal surfaces; some of these features exhibit noteworthy interspecific variations that are significant from a taxonomical standpoint (Mubo et al. 2004).

Essential diagnostic features of the epidermis that offer helpful suggestions for identification include size, stomata orientation, stomata form, guard cell shape, and structural features of epidermal cells (Munir et al. 2011; Trofimov and Rohwer 2018). In a related study, it was shown that trichome size and shape, stomata size and form, and the presence or absence of stomatal clusters were useful diagnostic traits for differentiating distinct fern species (Rahman et al. 2017).

Although it is now thought that epidermal traits are important for taxonomy, there is little information on the epidermal morphology of *Ficus* (Hussain et al. 2025). Notable characteristics such as epidermal cell structure, shape, orientation, stomatal complex size, and trichome types have major taxonomic implications (Rahman et al. 2017).

Leaf is the source tissue where the biosynthesis of majority of medicinally and economically important compounds takes place. *Ficus* is an important genus known for the synthesis of quite a few metabolites of medicinal value (Devi et al. 2022), which may be associated to the metabolite profiles. Thus, the current study was conducted to gain a comprehensive understanding of the importance of leaf epidermal structure in the identification of *F. elastica*, *F. elastica* and *F. natalensis*. This study will add to the existing literature on the use of leaf epidermal architecture in plant identification and provide insights for researchers and practitioners in plant taxonomy and categorisation.

## MATERIALS AND METHODS

An experiment was conducted in order to explore stomatal modifications in lower leaf lamina, petiole length and leaf surface area of different *Ficus* species in November, December and January. The leaves were collected from the New Botanical Garden, University of Agriculture, Faisalabad. Samples were preserved in FAA (formaldehyde-acetone-alcohol) fixative for 24 hours and then shifted to 70% ethanol for preservation. The hand sectioning of the leaf

lamina was performed. The leaf lamina was peeled off from the selected leaf samples and immediately placed in 30% ethanol for 10–15 minutes. These tissues were shifted to 50% ethanol, followed by 70% ethanol for 10–15 minutes, respectively. After 70% ethanol treatment, a few drops of safranin were added for 5 minutes. Safranin with 90% ethanol was used for 5 minutes. Then samples were washed 2–3 times with 100% ethanol. The samples were treated with 25%, 50% and 100% xylene for clearing them. Each section was permanently preserved in a drop of Canada balsam. The images of the samples were taken under an electron microscope.

## RESULTS

### *Length of petiole*

The comparison of different *Ficus* species petiole length recorded at different intervals (November, December and January) showed significant variations. Data showed a gradual increase in the petiole length of *F. elastica* over time. In contrast, *F. natalensis* consistently exhibited the minimum petiole length throughout all the experimental months, whereas other *Ficus* species showed no specific pattern in increase or decrease in length (Fig. 1A).

### *Leaf area*

Results revealed that *F. elastica* var. variegata leaf area was decreased from November to December and again increased from December to January. The *F. natalensis* had the smallest leaf area but remained constant throughout all the experimental months (Fig. 1B).

### *Number of stomata*

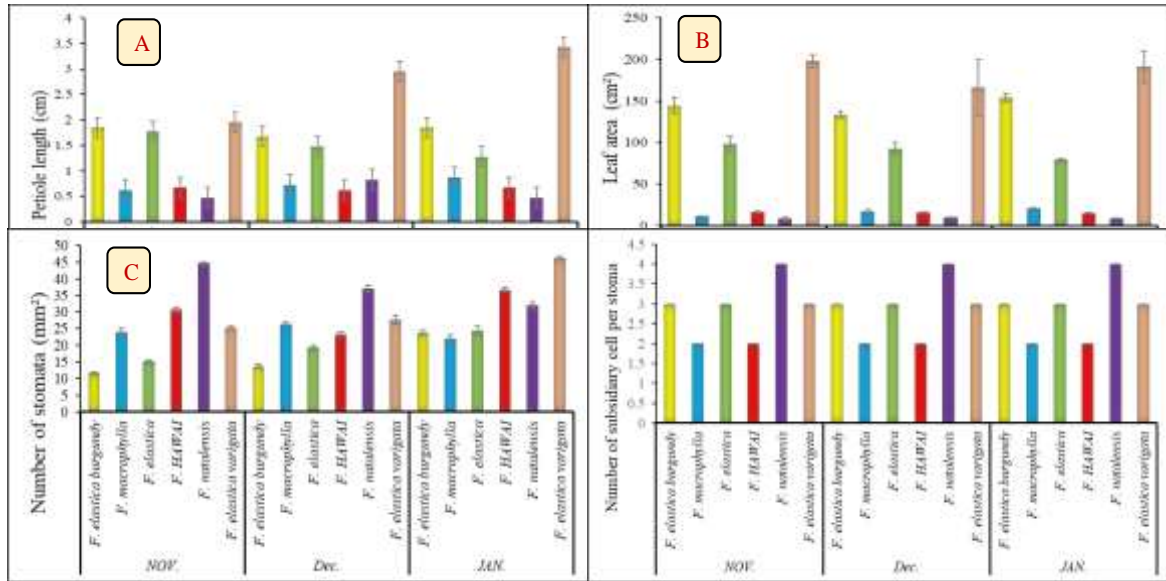
The comparison of *Ficus* species leaves epidermis collected at different time intervals (dawn, dusk, mid-day) in winter months revealed that there's a subsequent increase in the opening of stomata during mid-day. Among the species studied, *F. natalensis* had the maximum no. of stomata in November and December, but in January, *F. elastica* var. variegata had the maximum no. of stomata. Moreover, *F. elastica* 'Burgundy' had the least no. of stomata in almost all data taking months (Fig. 1C).

### *Number of subsidiary cells*

Data showed that *F. natalensis* showed the maximum no. of stomata and *F. hawaii* and *F. macrophylla* had the least no. of subsidiary cells (Fig. 1D).

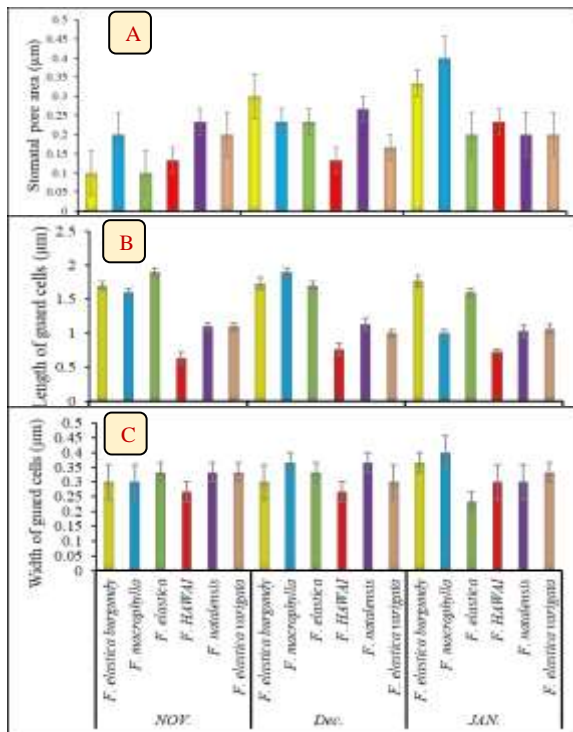
### *Stomatal pore area*

Results showed that the stomatal pore size of *F. macrophylla* and *F. elastica* 'Burgundy' gradually



**Fig.1:** Petiole length (A), Leaf area (B), number of stomata (C) and number of subsidiary cells of different *Ficus* species during different winter months.

increased across the observation periods, while *F. natalensis* pore size increased from November to December and then decreased from December to January



**Fig. 2:** Stomatal pore size (A), length of guard cells (B), and width of guard cells (C) of subsidiary cells of different *Ficus* species during different winter months.

(Fig. 2A–4).

#### Length of guard cells

The comparison of different *Ficus* species based on the length of guard cells recorded at different intervals in winter months (November, December and January) showed subsequent variations (Fig. 3–4). Results showed that the length of the guard cells of *F. elastica* gradually decreased over time, while the length of the guard cells of *F. hawaiiensis* increased across the observation periods (Fig. 2B).

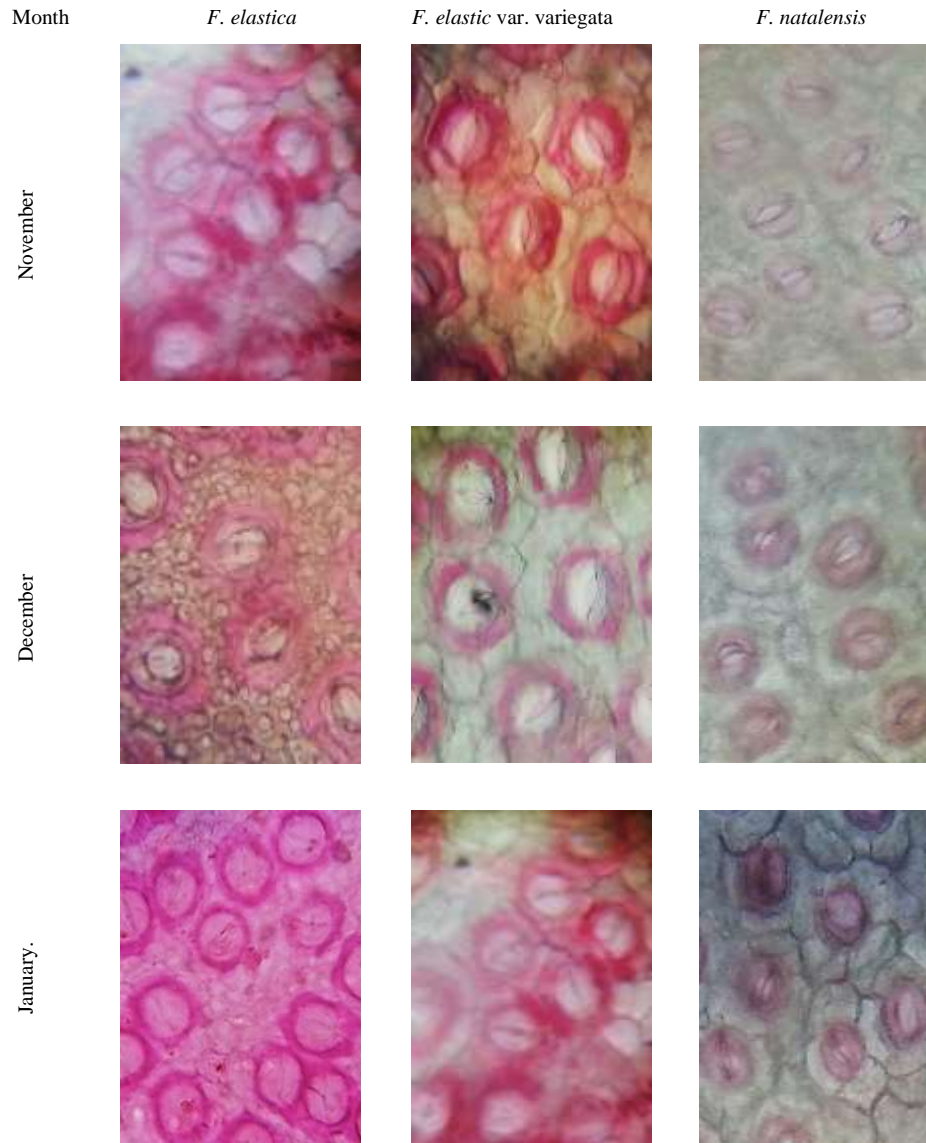
#### Width of guard cell

The results for guard cell width showed a gradual increase over time in *F. macrophylla*, whereas in *F. elastica*, guard cell width decreased with the passage of time (Fig. 7).

## DISCUSSION

The genus *Ficus*, commonly referred to as fig, belongs to the family Musaceae. *Ficus* is one of the largest plant genera, with more than 750 described species distributed worldwide, mainly in tropical countries. Fig trees are often ecologically important ‘Keystone’ components of tropical forests, because of the large number of vertebrates that feed on their figs, more than any other group of plants (Hussain *et al.* 2025).

A study was conducted to compare different morphological and anatomical characteristics of *Ficus* species. The result revealed that *F. elastica* had the maximum number of stomata, and the least were found in *F. natalensis*. The petiole length of *F. elastica* var. *variegata* increased over time. Over time, plants evolved different mechanisms to optimise their stomatal density in response to environmental



**Fig. 3:** Anatomical variations in stomatal complex of different *F. elastica*, *F. elastica* ‘Burgundy’ and *F. natalensis* during different winter months

cues to enhance their survival and fitness (Hou *et al.* 2023) (Fig. 1–2). Moreover, *F. natalensis* had shorter petiole length and showed no specific pattern in the increase and decrease in length during the observation period. The leaf area of *F. elastica* var. *variegata* decreased from November to December, and again, increased from December to January. In contrast, Shrestha *et al.* 2025) reported non-significant changes in the leaf area of *F. religiosa* during the winter season. In contrast, *F. natalensis* had the smallest leaf area, and no variation was marked from November to January. Furthermore, *F. natalensis* had the maximum number of stomata, and *F. hawaii* and *F. macrophylla* had the least number of subsidiary cells. Stomatal pore area of *F. macrophylla* and *F. elastica* ‘Burgundy’ gradually increased

with the passage of time, while *F. natalensis* pore area increased from November to December and then decreased from December to January (Fig. 3–4). Stomatal pore size or movement follows circadian rhythm, which is influenced by light exposure and the plants internal clock (Hou *et al.* 2023).

The length of the guard cell of *F. elastica* gradually decreased over time, while in *F. hawaii* it increased from November till January. In addition, the width of the guard cells of *F. macrophylla* gradually increased across the observation period, and in *F. elastica* the guard cell width decreased, respectively (Fig 1–4). Plants are affected by the environmental conditions during all phases of growth and development. Especially, stomatal number reportedly





**Fig. 4:** Anatomical variations in stomatal complex of different *F. hawaii*, *F. macrophylla* and *F. variegata* during different winter months.

changes when plants are grown in different seasons. Although the measurements were done on an area basis, this approach does not account for possible anatomical changes during different environmental conditions. It seems likely that the stomatal pattern may operate and respond to a range of conditions that can be explored in further studies. Stomata frequency declined in response to increasing CO<sub>2</sub> and may have occurred over geological time (Hofmann *et al.* 2025).

## CONCLUSIONS

changes in the number of epidermal cells, such as the number of stomata and subsidiary cells of *Ficus* species, were greatly affected by the environmental and climatic changes. These differences highlight the adaptive responses of each species to seasonal and diurnal changes. This study highlighted the

taxonomic importance of leaf epidermal features, emphasising their effectiveness as diagnostic traits for distinguishing among *Ficus* species. Future research should focus on the molecular and physiological mechanisms underlying stomatal regulation and anatomical adaptations in *Ficus* species under varying environmental conditions. Long-term field studies and genetic analyses could provide deeper insights into their adaptive strategies.

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## AUTHOR CONTRIBUTIONS

Muhammad Zubair Akram: Topic decision in publication, journal approach, publication process, data defining, writing and data collection. Ali Nawaz: Data collection, writing of article

## CONFLICTS OF INTEREST

The authors affirm that they possess no conflicts of interest.

## DATA AVAILABILITY

The data will be made available on a fair request.

## ETHICS APPROVAL

Not applicable to this paper.

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