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PHARMACOGNOSTICAL STANDARDIZATION, PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT AND ANTIDEPRESSANT POTENCY OF *IRIS GERMANICA* L

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ABSTRACT

Background: The plant species *Iris germanica* L., belonging to the family Iridiaceae, is a notable collection of approximately 1800 species. It is classified under the genus Iris, which is recognised as one of the largest genera. **Objective:** This study aims to provide a comprehensive overview of the pharmacognostical studies, phytochemical investigation, and evaluation of antioxidant and antidepressant activity of Iris germanica L. **Materials and methods**: The rhizomes of *Iris germanica* were collected, subjected to shade drying, and subsequently pulverised. The powdered sample was then assessed for pharmacognostic parameters, including macroscopic and microscopic characteristics, physicochemical properties, and phytochemical analysis, utilising established methodologies. The antidepressant efficacy of different hydroalcoholic fractions was assessed using the forced swim test, tail suspension test, and water wheel models. The assessment of the antioxidant activity of fractions was conducted through the utilisation of the DPPH free radical scavenging assay and the reducing power method. **Results:** The analysis of the hydroalcoholic extract of Iris germanica rhizomes yielded several phytoconstituents, including tannins, flavonoids, terpenoids, and carbohydrates. The findings of the study on antidepressant activity indicated that the ethyl acetate fraction of the hydroalcoholic extract of Iris germanica exhibited significant levels of activity compared to other fractions. The utilisation of this screening procedure holds significant value in determining the presence of various phytoconstituents. Additionally, the observed antidepressant potential of *Iris germanica* positions it as a promising candidate for the development of herbal antidepressant medications. Furthermore, the extracts exhibit considerable antioxidant capacity. **Conclusion:** In conclusion, this study provides valuable insights into the microscopic characteristics, proximate analysis, and preliminary phytochemical screening of Iris germanica rhizomes. The data

collected from this study can serve as a reference point for quality control measures in the plant under investigation. Additionally, it can be utilised as a medicinal substance for the alleviation of various ailments. Moreover, it can be considered a valuable source of information in the establishment of pharmacognostic standards pertaining to the aspects of quality, purity, identification, and classification. Additionally, the study on antidepressants and antioxidants demonstrated that the extracts exhibit noteworthy potential in terms of both antidepressant and antioxidant properties.

Keywords: *Iris germanica*, Genus, Standardization, Identification, Phytochemical Screening

INTRODUCTION

Depression, classified as a mental health disorder, is distinguished by enduring emotions of sadness, diminished enjoyment or interest in activities, and a variety of physiological and cognitive manifestations. The aforementioned phenomenon is a multifaceted ailment that has a global impact, affecting a substantial number of individuals[1]. Depression, referred to as major depressive disorder in academic literature, is a widely observed mental health condition that has a profound effect on the overall well-being and quality of life of individuals[2]. The condition is distinguished by a persistent and all-encompassing state of low mood, accompanied by a diminished interest or enjoyment in activities that were previously pleasurable. Although it is common for individuals to experience occasional feelings of sadness and low mood, depression is characterised by its heightened intensity, prolonged duration, and negative impact on daily functioning[3]. Depression is a complex mental health condition that arises from a combination of various factors, encompassing genetic, biological, environmental, and psychological influences. Depression can be initiated or exacerbated by significant life events, such as experiencing trauma, the death of a loved one, encountering relationship challenges, or facing financial hardships[4]. The aetiology of this condition is believed to involve neurochemical imbalances, particularly pertaining to serotonin, norepinephrine, and dopamin[5].

Depression presents with varying manifestations in each individual, albeit commonly characterised by enduring emotions of sadness, hopelessness, or emptiness, alongside a diminished capacity for experiencing interest or pleasure in previously enjoyed activities. Additional symptoms may encompass alterations in appetite and weight, disruptions in sleep patterns, persistent fatigue, challenges in maintaining focus or making decisions, sensations of diminished self-worth or guilt, and recurring contemplations of mortality or self-harm[6]. The presence of these symptoms can have a substantial impact on an individual's social, academic, or professional functioning[7]. It is imperative to acknowledge that depression is a manageable ailment. The treatment of depression typically encompasses a multifaceted approach that integrates psychotherapeutic techniques, pharmacological interventions, and modifications to one's lifestyle[8]. Cognitive-behavioral therapy (CBT) and interpersonal therapy (IPT) are widely employed therapeutic modalities that facilitate the identification and alteration of maladaptive cognitive processes, as well as the enhancement of adaptive coping mechanisms[9]. Antidepressant medications, such as selective serotonin reuptake inhibitors (SSRIs) or serotonin-norepinephrine reuptake inhibitors (SNRIs), may be prescribed to ameliorate symptoms and rectify neurochemical imbalances within the brain[10]. In addition, the presence of a supportive social network, engagement in regular physical exercise, sufficient sleep, and adoption of healthy lifestyle habits have been found to be influential factors in the comprehensive management and prevention of depression. Nevertheless, it is imperative to actively pursue expert assistance and guidance when confronted with depression, given its intricate nature that necessitates tailored interventions[11].

Despite the existence of efficacious interventions, depression continues to be a noteworthy matter of public health. The global prevalence of depression is estimated to affect more than 264 million individuals, positioning it as a prominent contributor to global disability. The presence of these comorbidities can exacerbate the effects of depression on an individual's life, necessitating comprehensive treatment strategies that encompass various dimensions of their overall well-being[12].

Iris germanica, commonly referred to as Juno in the English language and as Mazarmond in Kashmir region. The rhizome exhibits a substantial size, with extensive branching, a light brown coloration, and a uniform texture. The aerial stem measures 90 cm in height, surpassing the length of the leaves. It exhibits a characteristic of being covered with fine hairs, known as labrous, and possesses a bluish-green coloration, referred to as glaucous. Additionally, the stem maintains an upright and vertical orientation. The ovary exhibits a roughly triangular shape. The filaments measure 1.8 cm in length and exhibit a pale purple coloration, while the anthers are white and relatively similar in size. The dimensions of the capsule are approximately 3-5cm in length and 2.5cm in width. The period of flowering occurs between the months of April and June[13].

MATERIALS AND METHODS

The fresh rhizomes of the plant under study, *Iris germanica* L. were collected from Rangrate area of Budgam district, Jammu and Kashmir, India in the month of june-july (Figure 1). Prof. Akhtar H. Malik, curator at the University of Kashmir's Center for Biodiversity and Taxonomy, recognised and verified the specimen with voucher number 2230-KASH. For future use, a sample specimen of the obtained material was placed in the herbarium.

Chemicals and Reagents

The Standard drug Imipramine used in this study was procured from Ranbaxy lab as a gift sample, CMC and all other chemicals used were of analytical grade and were procured from registered dealers like Sigma Aldrich, CDH, S.D. Fine Chemicals Ltd., etc.

Macroscopical and Microscopical Evaluation

The macroscopic and microscopic analyses of *Iris germanica* L were performed following the methodologies described in the reference book "Trease and Evans Pharmacognosy."[14] The primary aim of this research was to examine strategies aimed at mitigating adulteration during the procurement phase of unprocessed medicinal substances obtained from *Iris germanica.*

Preparation of extracts

The fresh air-dried rhizomes of *Iris germanica* L. were powdered, filtered through 40 mesh, and then extracted with alcohol using cold extraction technique. A weighed quantity 500 gm of the powdered drug was extracted in a macerator with 70% ethanol. The hydroalcoholic extract was concentrated using rotary Vaccum evaporator under reduced pressure. After extraction, the dried hydroalcoholic extract was sequentially fractionated using Liquid-Liquid extraction process with different organic solvents such as Hexane DCM, Ethyl acetate and Butanol in the increasing order of polarity. The fractions were dried and then preserved in air tight glass containers for further use at 4°C.

Physicochemical analysis

The physicochemical analysis of *Iris germanica* L. rhizomes was carried out by determining extractive values, ash value, loss on drying, and pH of 1% and 10% solution[15]. Other parameters like swelling index, foaming index, were also determined[16].

Experimental animals

The study involved the procurement of Swiss albino mice, regardless of their sex, with a weight range of 25-30g from IIIM Jammu. These mice were obtained for the purpose of investigating the antidepressant efficacy of the fractions. The protocol for conducting the study on animals was approved by the institutional animal ethical committee under the approval number F (IAEC-Approval) KU/2018. The mice were housed in polypropylene cages and maintained under controlled environmental conditions, including a temperature of 25±2^{o}C, a 12:12 light: dark cycle, humidity levels ranging from 45-55%, unlimited access to water, and free availability of food.

Acute Oral Toxicity Study

The acute toxicity research was carried out in accordance with the Organisation for Economic Cooperation and Development (OECD) Guidelines No. 425. One animal was given an hydro alcoholic extract of Iris Germanic rhizomes at a dosage of 2000 mg/kg b.w, p.o. The animal was then closely monitored for a duration of 24 hours. In order to assess the viability of this particular species, an additional four specimens were administered an equivalent dosage and subjected to a 24-hour monitoring period. All of the animals survived, showing that the LD50 for all of the extracts is larger than 2000 mg/kg[17]

Experimental design

The animals were divided into six groups with six animal in each group.

Group 1: CMC (0.5%) by oral route [control group]

Group 2: Imipramine 15 mg/kg, IP) [Standard group].

Group3: Hexane fraction of the hydroalcholic extract of rhizomes of *Iris germanica* (50mg/kg, in 0.5% CMC by oral route) [IGH].

Group 4: DCM fraction of the hydroalcholic extract of rhizomes of *Iris germanica* (50mg/kg, in 0.5% CMC by oral route) [IGD].

Group 5: Ethyl acetate fraction of the hydroalcholic extract of rhizomes of *Iris germanica* (50mg/kg, in 0.5% CMC by oral route) [IGE].

Group 6: Butanol fraction of the hydroalcholic extract of rhizomes of *Iris germanica* (50mg/kg, in 0.5% CMC by oral route) [IGB].

Evaluation of antidepressant activity

Forced Swim Test (FST)

To carry out this experiment, procedure described by Porsolt et al. with slight modification was followed[18]. Briefly mice of either sex weighing 25-30 g were kept in an open tubular vessel with a waterwheel positioned across its centre, having dimensions of 10 cm in diameter and 25 cm height having 15 cm water at 25 ± 1^0C. The period of visible immobility was measured during the final 4 minutes of the 6-minute testing session.[19, 20] Animals were individually forced to swim in a confined environment from which they are unable to escape, inducing a typical behaviour of immobility. Each mouse was deemed immobile when it stopped fighting and stayed stationary in the water, making only the movements required to maintain its head above water. The length of immobility during the FST was used as an indicator of antidepressant efficacy. This process was continued for 14 days, with observations made on the first, seventh, and fourteenth days.

Tail Suspension Test (TST)

The method described by Steru et al. with slight modification was followed to carry out this procedure[21]. After treating animals with different test doses, the animals were individually hanged 50 cm above the ground by means of sticky tape positioned about 2 cm from the tip of the tail. During a test period of 6 minutes, the amount of time the mice were motionless was measured. Only when they hung passively and without making any movement were mice regarded to be immobile[22]. This process was continued for 14 days, with observations made on the first, seventh, and fourteenth days.

Statistical analysis: All values are presented as mean. One way ANOVA was used to assess the data, followed by the Dunnet Multiple comparison test.

Quantitative phytochemical analysis

Determination of Total phenolic content (TPC)

The total phenolic content in various fractions was determined using the standard Folin-Ciocalteu technique with minor modifications. Individually, the crude fractions were dissolved in methanol at a concentration of 1mg/mL. 0.5 mL of this methanolic solution was mixed with 2.5 mL of 10% Folin-

Ciocalteu reagent and 2.5 mL of 7.5% NaHCO3 to make the reaction mixture. The samples were then incubated in the dark for 45 minutes at 40°C. Finally, the absorbance at 765nm was measured in comparison to the blank, which is the reaction mixture without extract. The samples were prepared in triplicate for each assay, and the mean absorbance value was obtained. Gallic acid was employed as a control, and the findings were represented in gallic acid equivalents[25].

Determination of Total Flavonoid Content (TFC)

The content of total flavonoids in the various fractions was determined using the spectrophotometric method as described Quettier-Deleu et al. with slight modification. Briefly fractions were individually dissolved in methanol at concentration of 1 mg/mL. Reaction mixture was prepared by mixing 500µL of extract with 1mL of 2% $AlCl_3$ solution dissolved in methanol and then incubated at room temperature for one hour. Using a spectrophotometer, the sample was analyzed at 415nm. For each analysis triplicate samples were prepared. Same procedure was followed for standard drug rutin and the results were expressed in terms of rutin equivalent (RE/g of extract)[26].

***In vitro* Antioxidant activity determination**

Reducing power

The reducing power of the various fractions was estimated by the method described Prince et al. with minor modifications[25]. Various concentrations of *Iris germanica* fractions (100-500µg/mL) were prepared and were mixed with 2.5mL of 0.2M phosphate buffer having pH 6.6 and 2.5 mL of 1% potassium ferricyanide. Ascorbic acid was used as the standard in this method and were prepared in a similar manner. The reaction mixture was incubated at 50°C for 20 minutes. Following the incubation period, 2.5 mL of 10% trichloroacetic acid was added to the mixture. The whole reaction mixture was then centrifuged for 10 minutes at 3000 rpm. The top layer of solution (2.5 mL) was combined with 2.5 mL of purified water and 0.5 mL of 0.1% FeCl3. The blank reagent was made in the same way as described above, but without the addition of extract. At 700 nm, the absorbance was measured spectrophotometrically against a blank sample. Greater reducing power was demonstrated by increased absorbance of the reaction mixture.

DPPH free radical scavenging activity

DPPH assay of the various fractions was estimated by the method described by Braca et al. with slight modification[27]. Briefly, Fractions at varying concentrations (5 µg/mL to 100 µg/mL) were added to the freshly prepared DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in test tubes. For 30 minutes, the reaction mixture was incubated at room temperature. After incubation, the absorbance was measured using a spectrophotometer at 517 nm. Ascorbic acid was utilised as a standard. A control sample with the same volume but no extract or standard was created. Methanol was used as a blank[28].

RESULTS

Macroscopical Evaluation

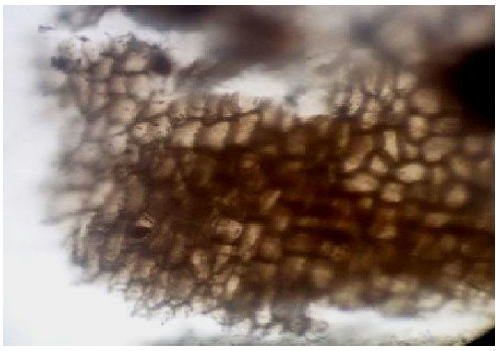
Characteristics	**Observation**
Colour	Brown (Externally), Yellowish (internally)
Odor	Characteristic
Taste	Bitter
Shape	Irregular
Size	5-6 cm long, 1.5-2.5 cm wide, 1.0-2.0 cm
Texture	Fibrous and smooth

Figure 1: Rhizomes of *Iris germanica* L.

Powder Microscopy of the rhizomes of *Iris germanica* L.

The colour of powdered rhizome is brown, odor is characteristic, and its taste is bitter. Powder microscopy of rhizomes revealed the presence of a). Prismatic calcium oxalate crystals; b). Cork cells; c). Lignified Xylem vessels d) Starch granules;

a) Prismatic calcium oxalate



b) Cork cells

c) Lignified Xylem vessels

d) Starch granules

Figure 2: Powder Microscopy of the rhizomes of *Iris germanica* L.

Physicochemical Constants the rhizomes of *Iris germanica* L

Physicochemical parameters viz. total ash value, acid-insoluble ash value, sulphated ash value, loss on drying, foaming index, swelling index, foreign matter, pH, and extractive values were evaluated and the results are tabulated in **Table 1**. For fluorescence analysis the powdered drug was treated with different reagents and analysed under visible and UV light. The behavior of the powdered drug and

the colors imparted on treatment with different reagents were studied and the results are given in **Table 2.** The content of the heavy metals was determined which passed the permissible limits as per WHO, 1998 guidelines and the results are given in **Table 3**. Various fractions isolated from hydroalcoholic extract were subjected for phytochemical analysis and the results are given in **Table 4.**

Table 1: Proximate analysis and extractive values of *Iris germanica* L.

Physicochemical parameters	**Results**	
Total ash value	9.235	
Acid insoluble ash value	2.800	
Sulphated ash value	1.043	
Water soluble ash value	3.0025	
Loss on drying	18.0666	
Foaming index	Less than 100	
Swelling index	3	
Foreign matter	0.016	
pH of 1% solution	6.3	
pH of 10% solution	6.1	
Extractive values	Cold extractive value	Hot extractive value
Ethanolic	9.718	11.37
Aqueous	12.760	13.567
Hydroalcholic	14.23	21.56

Table 2: Fluorescence analysis of powdered drug of *Iris germanica* with various reagents under visible light, short and long wave length*.

Drug Treatment	**Visible light**	**Short UV (254nm)**	**Long UV (366)**
Powder drug as such	Light Brown	Green	Green
Powder drug + Dist. Water	Buff	Vivid Green	Green
Powder drug + Conc. HCL	Brown	Green	Green
Powder drug + Dil. HCL	Brown	Brown	Green
Powder drug + H_2SO_4	Blackish Brown	Dark Green	Black
Powder drug + Dil. H_2SO_4 (10%)	Reddish Black	Green	Black
Powder drug +HNO_3	Brown	Black	Green
Powder drug + Dil. HNO_3 (10%)	Yellow	Brown	Green
Powder drug + 10% NaOH	Brown	Green	Green
Powder drug + picric acid	Yellow	Black	Green
Powder drug + Iodine	Brown	Black	Black
Powder drug + Methanol	Yellow	Espresso	Black
Powder drug + Ethanol	Yellow	Brown	Green
Powder drug + acetic acid	Brown	Green	Green
Powder drug + Chloroform	Brown	Brown	Vivid Green
Powder drug + Pet. ether	Brown	Brown	Green
Powder drug + Ferric chloride	Black	Black	Black
P. drug + Ammonia solution	Buff	Brown	Green

Table 3: Heavy metal analysis of the rhizomes of *Iris germanica* L.

Sl. No.	**Test Parameters**	***Iris germanica* (ppm)**	**MDL(WHO) (ppm)**
1.	Cadmium (Cd)	0.035	0.3
2.	Chromium (Cr)	0.053	2.0
3.	Nickel (Ni)	0.074	0.63
4.	Lead (Pb)	0.216	1.0
5.	Mercury (Hg)	0.045	0.1

Table 4: Phytochemical screening of different extracts of the rhizomes of *Iris germanica* (IG).

Tests	IGHa	IGH	IGD	IGE	IGB	IGRaq
Tests for Carbohydrates						
Molisch's test	++	-	+	++	++	++
Fehling's test	+	-	+	++	++	++
Benedict's test	+	-	+	++	+	+
Tests for Tannins						
5% FeCl3 test	++	+	++	++	++	+
Lead acetate test	+	++	++	+	+	++
Tests for Phenolics						
1% FeCl3 test	++	+	++	++	++	+
Tests for Flavonoids						
Shinoda test	+	-	+	+	+	-
Tests for Saponins						
Foam test	+	+	+	+	+	+
Froth test	+	+	+	+	+	+
Tests for Terpenoids						
Salkowski test	++	-	++	++	+ +	-
Tests for Phytosterols						
Libermann's test	+	-	+++	+++	+++	+
Tests for Proteins and Aminoacids						
Xantho-proteic test	-	-	-	-	-	-
Ninhydrin test	-	-	-	-	-	-
Tests for Cardiac glycosides						
Keller killiani test	+	+	++	+++	+++	-
Legal test	+	+	+	++	++	-
Tests for Alkaloids						
Mayer's test	-	-	-	-	-	-
Hager's test	-	-	-	-	-	-
Dragendroff's test	-	-	-	-	-	-
Wagner's test	-	-	-	-	-	-

+ (Present); - (Absent)

IGHA (*Iris germanica* hydroalcoholic), IGH (*Iris germanica* hexane fraction), IGD (*Iris germanica* dichloromethane fraction), IGE (*Iris germanica* ethyl acetate fraction), IGB (*Iris germanica* n-butanol fraction), IGRaq (*Iris germanica* residual aqueous fraction).

Acute oral toxicity results

On acute oral toxicity no lethal effects were seen and all animals used in the study survived, which indicted that LD50 is greater than 2000mg/kg bw for all the fractions. Therefore, for further experimental evaluation 50 mg per kg bw was selected on the basis of acute toxicity study.

Evaluation of antidepressant activity

In order to evaluate the antidepressant potency of various fractions of *Iris germanica* rhizomes, forced swimming test and tail suspension test models were utilized. The animals treated with the various fractions of rhizomes showed prominent antidepressant potency in both the models. The data obtained revealed that ethyl acetate prominently reduce immobility time in both FST and TST followed by n-butanol and dichloromethane fraction. In FST it was also that revealed that ethyl acetate prominently reduces number of rotations as compare to control group followed by n-butanol and dichloromethane fraction. The results of FST, effect on number of rotations and TST are given in Table-5-7 and figure: 3-5.

Table 5: Forced Swimming Test results of various fractions of *Iris germanica*

Groups	Dose (mg/kg)	Duration of immobility		
		DAY1	DAY7	DAY14
Control	**CMC 0.5%**	117.00±3.324	111.00±2.765	108.00±3.456
Imipramine	**15mg/kg**	81.50±1.863*	67.40±2.443**	44.65±2.098***
IGH	**50mg/kg**	115.00±4.974ns	113.00±3.423ns	111.76±3.90*
IGD	**50mg/kg**	100.63±1.345*	97.35±1.098*	83.66±2.456*
IGE	**50mg/kg**	98.47±2.556*	94.30±2.432*	78.98±3.99**
IGB	**50mg/kg**	100.00±3.642*	97.66±4,764ns	80.67±2.921*

Results were expressed as mean ± SEM n =6 rats ***P < 0.001, **P < 0.01, *P < 0.05. nsP>0.05 will be considered extremely significant, highly significant, significant and insignificant respectively compared with control. Data was statistically analysed by One way ANOVA followed by Dunnet Multiple comparison test.

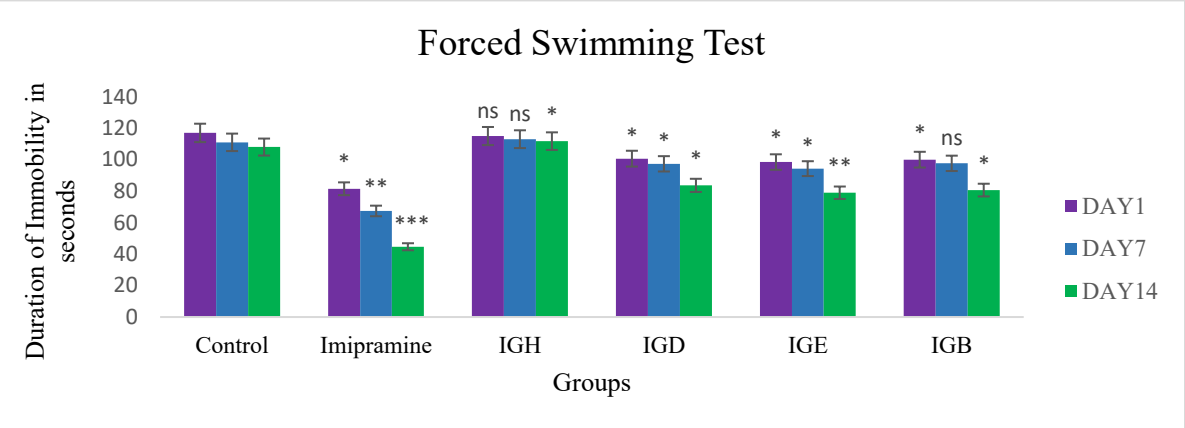


Figure 3: Graphical representation of Forced Swimming Test of various fractions of *Iris germanica*

Table 6: Effect on number of rotations by various fractions of *Iris germanica*

Groups	Dose (mg/kg)	Number of Rotations		
		Day 1	Day 7	Day 14
Control	**CMC 0.5%**	19.700±2.17	20.34±2.21	22.21±2.98
Imipramine	**15mg/kg**	55.650±4.93**	57.00±3.09**	60.98±4.65**
IGH	**50mg/kg**	21.668±2.61ns	22.04±3.23*	25.98±2.25*
IGD	**50mg/kg**	32.000±3.13*	35.67±4.87*	40.32±3.43*
IGE	**50mg/kg**	28.000±4.67**	29.00±3.54*	33.00±3.09*
IGB	**50mg/kg**	25.450±2.87*	27.00±3.67*	30.44±2.65*

Each value is mean ± SEM n =6 rats ***P < 0.001, **P < 0.01, *P < 0.05. nsP>0.05 will be considered extremely significant, highly significant, significant and insignificant respectively compared with control. Data was statistically analysed by One way ANOVA followed by Dunnet Multiple comparison test.

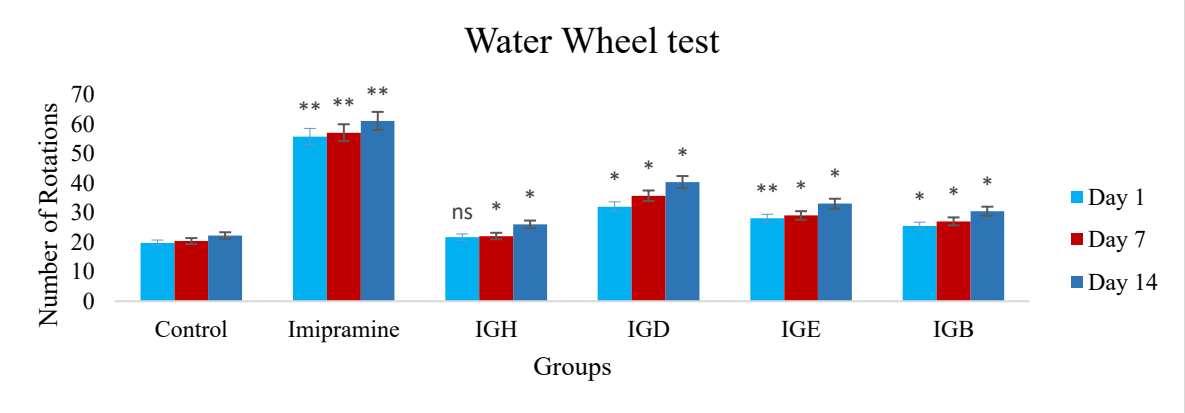


Figure 4: Graphical representation of effect of various fractions of *Iris germanica* on number of rotations.

Tail suspension test

The immobility time of the different groups of mice is shown in the table given below:

Table 7: Shows Tail Suspension Test results of various fractions of *Iris germanica*

Groups	Dose (mg/kg)	Duration of immobility		
		DAY1	DAY7	DAY14
Control	**CMC 0.5%**	115.30±7.034	111.00±6.745	109.00±4.675
Imipramine	**15mg/kg**	53.20±5.506**	40.90±5.078**	24.30±5.252**
IGH	**50mg/kg**	106.00±4.143*	99.00±6.223ns	92.56±4.678*
IGD	**50mg/kg**	67.76±5.098**	65.89±5.477**	57.43±3.678ns
IGE	**50mg/kg**	59.07±2.876ns	52.39±3.98*	47.87±2.378*
IGB	**50mg/kg**	100.36±3.98ns	87.90±2.743*	74.80±3.234*

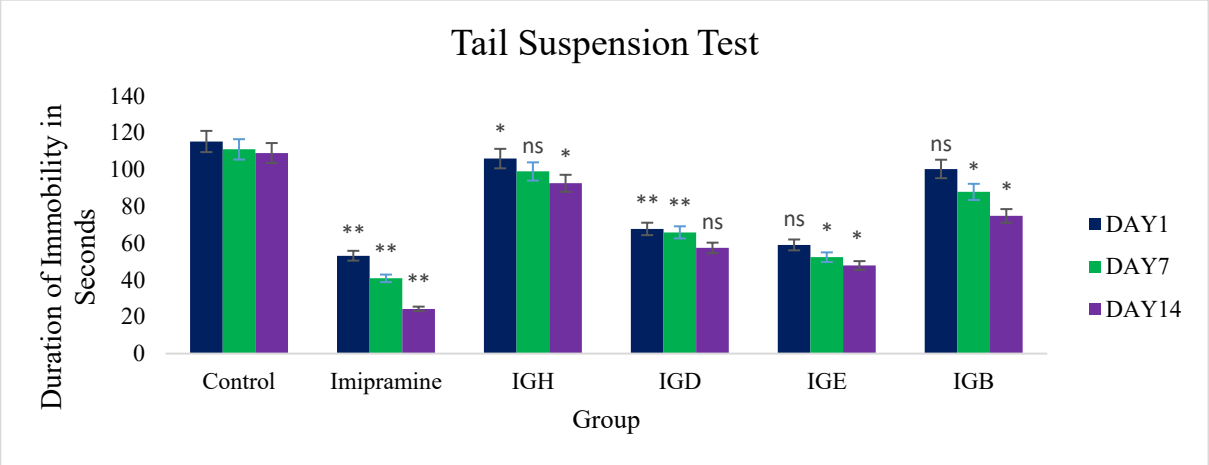


Figure 5: Graphical representation of Tail Suspension Test of various fractions of *Iris germanica*

Each value is mean ± SEM n =6 rats ***P < 0.001, **P < 0.01, *P < 0.05. nsP>0.05 will be considered extremely significant, highly significant, significant and insignificant respectively compared with control. Data was statistically analysed by One way ANOVA followed by Dunnet Multiple comparison test**.**

Total phenolic and flavonoid content of *Iris germanica*

The results of total phenolic content and total flavonoid content were given table 9 and figure 5. Various fractions isolated from *Iris germanica* rhizomes showed good amount of total phenolic and flavonoid content ranging from 21.64 to 307.45 mg GAE/g dry weight and 15.86 to 287.54 mg RE/g dry weight of the sample respectively. The highest TPC and TFC was found in ***IGE*** **(**307.45 and 287.54**)** followed by ***IGD*** (253.15 and 119.42) and **IGB** (182.38 and 45.88) respectively. ***IGH*** showed the least total phenolic content (21.64) and total flavonoid content (15.86).

Table 8: Total Phenolic Content and Total Flavonoid Content of various extracts of *Iris germanica*

Extract	Total Phenolic Content (mg GAE/g of extract)	Total Flavonoid Content (mg RE/g of extract)
IGHa	143.37 ± 7.07	121.66 ± 4.22
IGH	21.64 ± 6.30	15.86 ± 5.76
IGD	253.15 ± 3.32	119.42 6.01
IGE	307.45 ± 8.25	287.54 ± 8.54
IGB	182.38 ± 4.71	45.88 ± 3.61
IGRaq	115.721 ± 11.19	20.86± 0.45

The values are represented as mean±SD; (n=3)

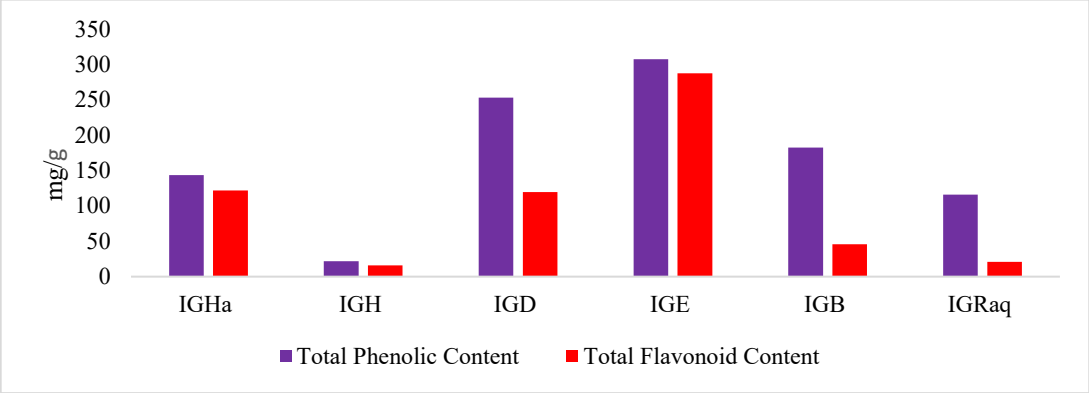


Figure 6: Total Phenolic and Flavonoid contents of *Iris germanica*

In-vitro antioxidant screening

Reducing power:

All the fractions isolated from *Iris germanica* rhizomes showed potent reducing power potency. The highest potency was shown by ethyl acetate fraction (3.231), followed by dichloromethane fraction (1.987), n-butanol fraction (1.432) at 500 µg/mL. Standard ascorbic acid showed reducing power of 4.112 at the same concentration. Figure 8.

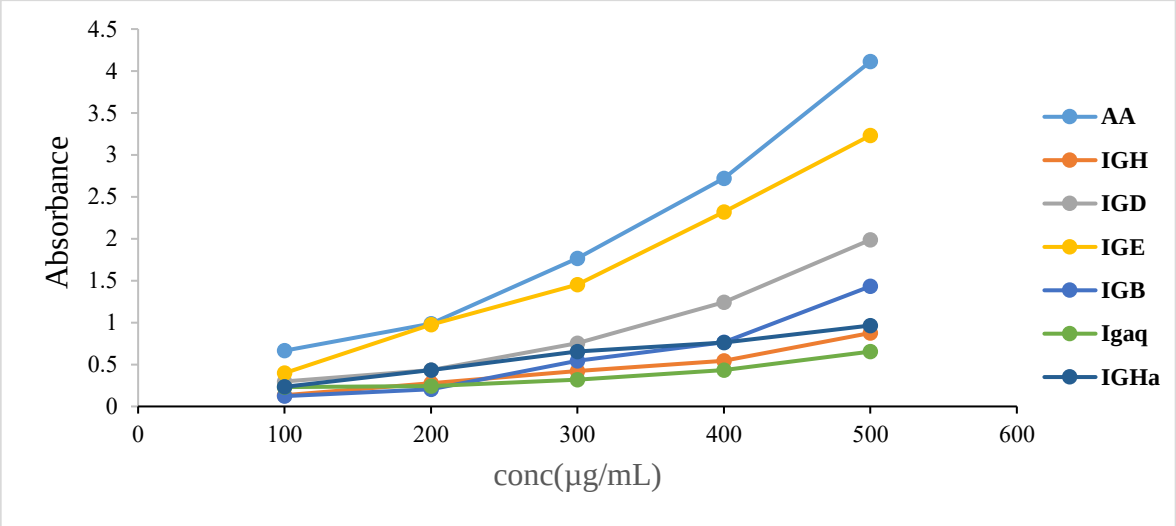


Figure 7: Reducing power of *Iris germanica* fractions

DPPH scavenging assay

All the fractions isolated from *Iris germanica* rhizomes showed potent DPPH quenching potency. The highest potency was shown by ethyl acetate fraction with IC50 value of 33.79, followed by dichloromethane fraction with IC50 value of 60.64. Standard ascorbic acid showed IC50 value 8.27 under similar conditions. Table 9

Table 9: Antioxidant potential in terms of IC50 (µg/mL) of various extracts of *Iris germanica*

Sample	DPPH (IC50)
AA	8.27± 0.52
IGHa	109.74± 1.10
IGH	150.28 ± 8.80
IGD	60.64 ± 2.02
IGE	33.79± 3.03
IGB	101.09 ± 1.775
IGRaq	472.62 ± 8.76

Discussion

The present investigation elucidates the pharmacognostic, phytochemical, microscopic, and macroscopic characteristics of the rhizomes of Iris germanica. This investigation aims to facilitate the accurate identification of the rhizome, thereby enabling the preparation of a crude extract. This study is regarded as the preliminary measures for the quality control and authentication of herbal drugs in accordance with the guidelines established by the World Health Organisation (WHO). The current examination of the rhizomes of Iris germanica, conducted at both microscopic and macroscopic levels, provides valuable insights into the quality control parameters of the crude drug. The detection of foreign material, such as soil and sand, in the powdered drug was conducted through the determination of ash value. The quantification of active constituents was accomplished through the acquisition of extractive values. The determination of the volatile content and moisture content in the sample was conducted using the loss on drying method. The pH of the sample was analysed to determine the concentration of acidic and basic compounds present. Fluorescence analysis was conducted in order to ascertain the presence of fluorescent components, as this parameter holds significant importance in pharmacognostical evaluation. The hydroalcoholic extract of the rhizomes of Iris germanica was subjected to phytochemical screening, which identified the presence of several phytoconstituents including tannins, flavonoids, terpenoids, carbohydrates, and others. During the assessment of antidepressant activity, it was noted that the ethyl acetate extract derived from the plant being investigated exhibited a noteworthy decrease in immobility time compared to the standard. Additionally, the water wheel experiment indicated an decrease in the number of rotations. The findings from the in vitro antioxidant assays demonstrated the strong antioxidant capacity of various IG extracts. The *Iris germanica* plant, which is native to the Kashmir valley, contains substantial quantities of flavonoids and phenolics. These phytoconstituents are of great importance due to their various biological activities, particularly their ability to prevent oxidative damage in vitro. The findings presented in this study offer empirical support for the notion that this particular plant possesses the potential to serve as a viable source of natural antioxidant agents.

CONCLUSION

Iris germanica L rhizomes possess prominent antidepressant effect in animal models of depression which was comparable to that of Imipramine as demonstrated in this study. The phytochemical screening results revealed the presence of alkaloids, flavonoids, diterpenes, proteins, amino acids, tannins, saponins, phytosterols, and phenolic compounds in the crude extract.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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