RESEARCH PAPER

Evaluation of antioxidant and anti-fungal potential of leaves & flowers extract of Hippeastrum vittatum.

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ABSTRACT

Human beings have always involved themselves in various activities to ensure their wellbeing and survival. In doing so, the human body has directed the release of different free radicals or reactive substances which are either inhaled or consumed. The study was based on the evaluation of antioxidant and anti-fungal potential of leaves & flowers extract of Hippeastrum vittatum. The Fresh leaves and flowers of Hippaestrum vittatum were collected from UP East region and authenticated by a Scientist at BSI, Prayagraj. Wistar rats of either sex weighing 150-200g were obtained from the Animal House, School of pharmacy and medical sciences, Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan, India. Extraction of the leaves and flowers of Hippaestrum vittatum was done through cold maceration process using methanol and hydroalcoholic solvent, separately. All the animals were divided into 5 groups (n=6) and treated for 14 days i.e., group I administered carboxymethylcellulose sodium (0.5%); control group, group II administered 200mg/kg of methanolic leaves extract of H. vittatum, group III administered 200mg/kg of hydroalcoholic leaves extract of H. vittatum, group IV administered 200mg/kg of methanolic flowers extract of H. vittatum and group V administered 200mg/kg of hydroalcoholic flowers extract of H. vittatum. The antioxidant activity was evaluated using following parameters i.e., DPPH free-radical scavenging assay, SOD activity and assay of lipid peroxidation. Anti-fungal activity was evaluated through well diffusion method against following fungal strains i.e., Aspergillus niger, Penicillium notatum, Candida albicans and Rhizopus species. In results, methanolic flowers extract of H. vittatum (200mg/kg) and hydroalcoholic flowers extract of H. vittatum (200mg/kg) exhibited the TBARS level as 163.26±1.17 and 168.45±1.12, respectively. In conclusion, leaves extract of lily showed a moderate DPPH scavenging potential while flowers extract of H. vittatum (lily) exhibited a more significant DPPH scavenging potential which might be due to release of more phytochemical constituents in flowers extract. SOD and TBARS level were found modulated significantly by the flowers extract of H. vittatum (lily) in contrast to leaves extract of H. vittatum. Moreover, methanolic fractions reported a higher level of total phenolic and total flavonoids contents than hydroalcoholic fractions which might be due to better solubility of phytocomponents. Flowers extract of H. vittatum (200mg/kg) exhibited highest anti-fungal potential as A. niger which was near to standard anti-fungal (Itraconazole gel)..

Keywords: *Hippaestrum vittatum, antioxidant, anti-fungal, DPPH assay, anti-fungal activity...*

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1. INTRODUCTION

Human beings have always involved themselves in various activities to ensure their wellbeing and survival. In doing so, the human body has directed the release of different free radicals or reactive substances which are either inhaled or consumed [1]. There are microorganism's bacteria everywhere. They are essential to maintaining our living environment. Worldwide, just a small percentage of micro-organisms cause disease and infection. These bacterial and fungal illnesses have a major effect on the health of the general people [2].

The lily bulbs are tunicate, meaning they have mushy concentric inner scales or leaf bases and a protective, dry outer shell. Two to seven durable evergreen or deciduous leaves, measuring 30 to 90 cm in length and 2.5 to 5 cm in width, are produced by bulbs that are typically 5 to 12 cm in diameter. The leaves are sessile, hysteranthous, sub-petiolate, and infrequently persistent. Two free bracts form a bivalve spathe with free leaflets at its base, and the flowers are arranged in umbelliform inflorescences [3]. There are two to fifteen huge, spectacular flowers, which are more or less hermaphrodite and zygomorphic, depending on the species. The native species are often red or purple, with each blossom measuring 13 to 20 cm

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(5-8) for diameter. Their shapes are declinate (curving downward and then upward at the tip) and funnelform (funnel shaped). Three outer sepals and three inner petals make up the perianth's six vibrantly colored tepals, which can have a similar or drastically distinct look. The segments of the perianth are either uneven or subequal. The tepals are joined at the base to form a small tube, which typically has a callose ridge at the neck or a simple scaly paraperigonium with fimbriae [4][5].

Flower **b. Leaves**

Fig 1. *Hippeastrum vittatum* (Lily) plant

Taxonomy

Kingdom : Plantae

Order : Asparagales

Family : Amaryllidaceae

Genus : *Hippeastrum*

Species : *vittatum*

The ethanolic fresh flower extract from *Hippeastrum vittatum* revealed the unique alkaloids, tannins, glycosides, i.e., Ismine, Lycorine, Crinine, Vittacarboline, Haemanthamine, Narciclasine, Galanthamine and Tazettine [6]. The study was based on the evaluation of antioxidant and anti-fungal potential of leaves & flowers extract of *Hippeastrum vittatum.*

MATERIALS AND METHODS

Experimental requirements

Fresh leaves and flowers of *Hippaestrum vittatum*, methanol, ethanol, DPPH, distilled water, Wistar albino rats (either sex), rotatory evaporator and weighing machine.

Collection, authentication, and preparation of extract

The Fresh leaves and flowers of *Hippaestrum vittatum* were collected from UP East region and authenticated by a Scientist at BSI, Prayagraj. The leaves and flowers were washed making dust-free and dried at room temperature or shade. The dried leaves and flowers were rendered into coarse powders and then finally into fine ones. The powders of each were weighed and soaked in methanol and hydroalcoholic solvent (1:1), separately for fifteen days with gradual stirrings. The obtained slurry of mixture was kept for drying under partial vacuum using a rotary evaporator or water-bath [7].

Preparation of animals

Wistar rats of either sex weighing 150-200g were obtained from the Animal House, School of pharmacy and medical sciences, Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan, India. The animals were maintained in proper conditions, at room temperatures of 25±1°C with 12-hour light/dark cycle. The relative humidity was maintained at 44-56%, and animals fed with standard rodent pellet diet and water *ad libitum*. 01-hour prior experiment, rats are taken off their food.

Group design

All the animals were divided into 5 groups (n=6) and treated for 14 days as follows:

Group I administered carboxymethylcellulose sodium (0.5%); control group.

Group II administered 200mg/kg of methanolic leaves extract of *H. vittatum.*

Group III administered 200mg/kg of hydroalcoholic leaves extract of *H. vittatum.*

Group IV administered 200mg/kg of methanolic flowers extract of *H. vittatum.*

Group V administered 200mg/kg of hydroalcoholic flowers extract of *H. vittatum.*

Evaluation of antioxidant potential

Blood collection and preparation of tissue homogenate

Blood samples of the experimental animals were collected by retro orbital artery bleeding after 16 hr of the last dose. Blood samples were centrifuged for 10 minutes at 2000 rpm to separate the serum. The rats were sacrificed by diethyl-ether (anaesthesia) on day 8 and liver was excised, rinsed in 0.25 M sucrose solution and 10% w/v homogenate was prepared in 0.15M KCl, centrifuged at 1000 rpm for 10 min followed by centrifugation of the supernatant at 12000 rpm for 15 min. The supernatant obtained was further used for estimation of various oxidative enzymes.

Estimation of DPPH Free-Radical Scavenging Assay

The free radical scavenging potential of leaves and flower extracts of Lily were measured *in-vitro* by the 1,1′-diphenyl-1-picrylhydrazyl (DPPH) according to the method by Tariq et al. [8]. The assay experimented by reacting 1.6 mL of 0.135 mM DPPH dissolved in 100% v/v methanol with 0.4ml of various concentrations (0.0078–2mg/mL) of methanolic and hydroalcoholic leaves and flower extracts of Lily. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture measured at 517 nm after 2 min. Here, the concentration of the lily needed to decrease the absorbance of DPPH radical by 50% was calculated. Rutin (Sigma-Aldrich, ≥94%, HPLC grade) at the same working concentrations of the lily was used as reference drug.

The % scavenging activity was determined by the following equation:

DPPH scavenging activity (%) = [(control absorbance−sample absorbance)/ (control absorbance)]× 100

Estimation of Super Oxide Scavenging Activity (SOD)

The assay of SOD was based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome. To the supernatant, carbonate buffer and EDTA were added. The reaction was initiated by addition of 0.5ml of epinephrine and the auto-oxidation of adrenaline to adrenochrome at pH 10.2 was measured by following changes in optical density at 480nm. The changes in optical density every minute were measured at 480nm against a reagent blank. The results were expressed as units of SOD activity. One unit of SOD activity induced approximately 50% inhibition of adrenaline. The results were expressed as nmol SOD U per mg wet tissue [9].

Assay of lipid peroxidation

1ml of herbal extract was combined with 0.2 ml sodium dodecyl sulphate, 1.5 ml acetic acid in hydrochloric acid, and 1.5 ml thiobarbituric acid in hydrochloric acid. The resulting mixture was heated for 1 hour in a hot water bath at 85°C. At 532 nm, the intensity of pink colour generated was measured against a blank [10].

Evaluation of anti-fungal activity

By using the well diffusion method, the antifungal activity of methanolic and hydroalcoholic leaves and flower extracts of Lily against fungus were identified. Nutrient Agar broth was utilized to cultivate a 24-hour-old culture of fungus, which was then used to make a suspension of fungi. After sterilization at 121°C (1.05 kg/cm2 pressure) for 20 minutes, nutrient agar solution was added. A sterile spreader was used to cover the whole surface of the agar plates when we inoculated them with 500:l of each fungal suspension. With a sterile cork borer, 5mm wells were made in the solidified media, and each well was filled with methanolic and hydroalcoholic leaves and flower extracts of Lily. The diameter (mm) of the inhibitory zone surrounding the well was measured after 24 hours of incubation at 30°C. As a standard antifungal formulation, itraconazole gel (Itromed 1%) was utilized.

Every antifungal study was carried out in triplicate. Fungus strains i.e., *Aspergillus niger, Penicillium notatum, Candida albicans and Rhizopus species* were used for screening of anti-fungal activity [11].

RESULTS AND DISCUSSION

Percentage yield

The percentage yield was obtained as 63.47% and 58.26% in methanolic and hydroalcoholic leaves extract of *Hippaestrum vittatum* (Lily), respectively. However, the methanolic and hydroalcoholic flowers extract of *Hippaestrum vittatum* showed the percentage yield of 53.41% and 48.16%, respectively.

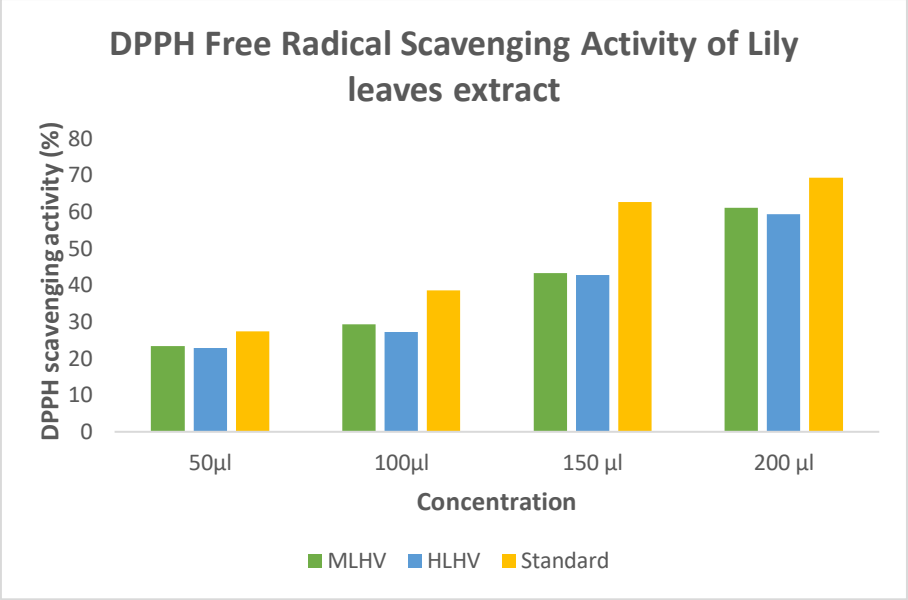
Evaluation of antioxidant activity

Estimation of DPPH Free Radical Scavenging Activity

The lily extract was determined for the DPPH radical scavenging potential at different concentrations i.e., 50µl, 100µl, 150µl, 200µl. At 200 µl, methanolic leaves extract of *H. vittatum* (MLHV), hydroalcoholic leaves extract of *H. vittatum* (HLHV) and standard group showed the scavenging activity as 61.14 %, 59.43 % and 69.24 % respectively.

Table 1. DPPH Free Radical Scavenging Activity of Lily leaves extract

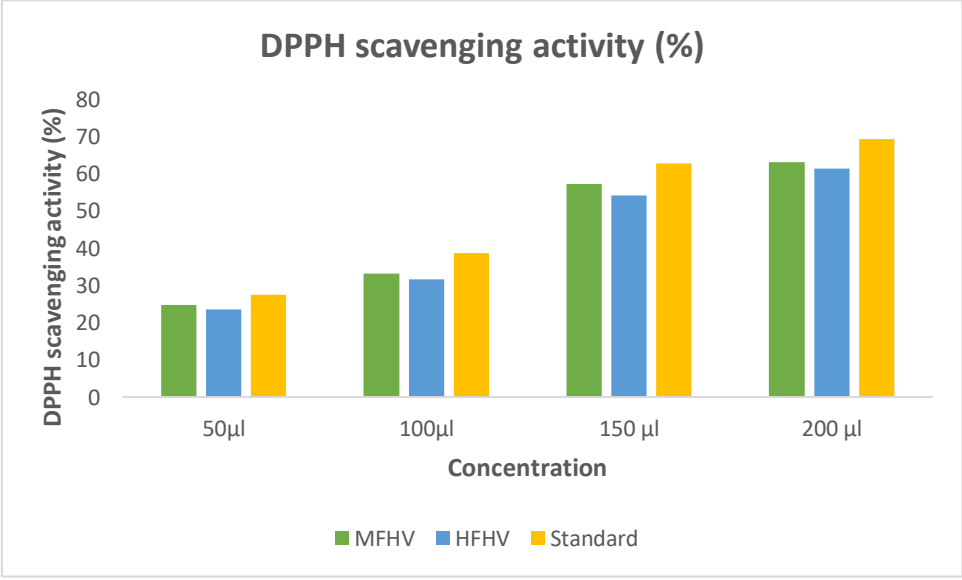
Concentration	DPPH scavenging activity (%)		
	MLHV	HLHV	Standard
50µl	23.46	22.83	27.41
100µl	29.34	27.14	38.62
150 µl	43.29	42.71	62.75
200 µl	61.14	59.43	69.24



Similarly, DPPH scavenging activity was determined for flowers extract of lily. At 200 µl, methanolic flowers extract of *H. vittatum* (MFHV), hydroalcoholic flowers extract of *H. vittatum* (HFHV) and standard showed the scavenging activity as 63.17 %, 61.43 % and 69.24 % respectively.

Table 2. DPPH Free Radical Scavenging Activity of Lily flowers extract

Concentration	DPPH scavenging activity (%)		
	MFHV	HFHV	Standard
50µl	24.81	23.47	27.41
100µl	33.17	31.68	38.62
150 µl	57.23	54.10	62.75
200 µl	63.17	61.43	69.24



Determination of SOD Activity

Methanolic leaves extract of *H. vittatum* (200mg/kg) reported the SOD level as 4.84±0.41. While highest SOD level was found in methanolic flowers extract of *H. vittatum* (200mg/kg) as 4.97±0.45 respectively.

Table 3. SOD activity of lily extract

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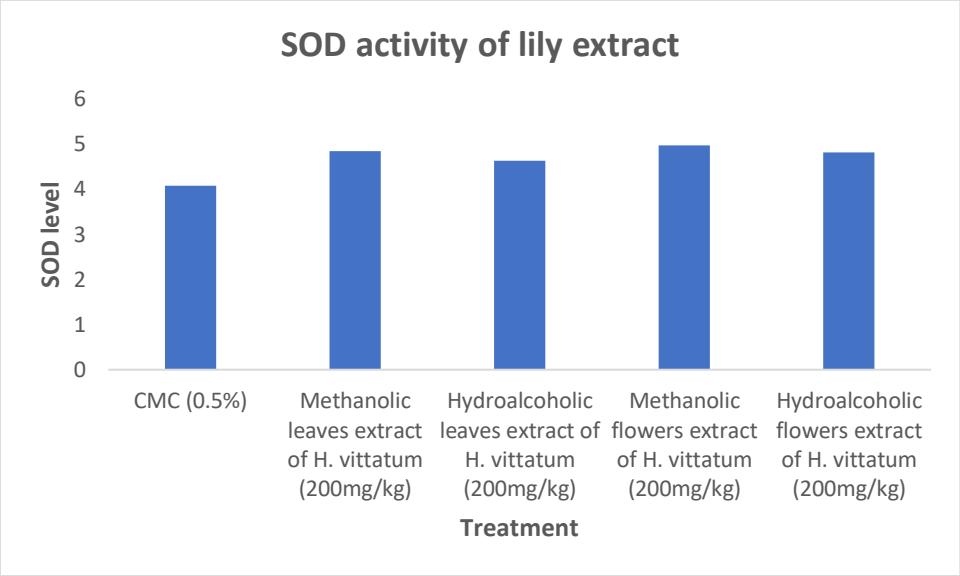
Treatment	SOD level
CMC (0.5%)	4.07±0.29
Methanolic leaves extract of *H. vittatum* (200mg/kg)	4.84±0.41
Hydroalcoholic leaves extract of *H. vittatum* (200mg/kg)	4.63±0.27
Methanolic flowers extract of *H. vittatum* (200mg/kg)	4.97±0.45
Hydroalcoholic flowers extract of *H. vittatum* (200mg/kg)	4.81±0.20

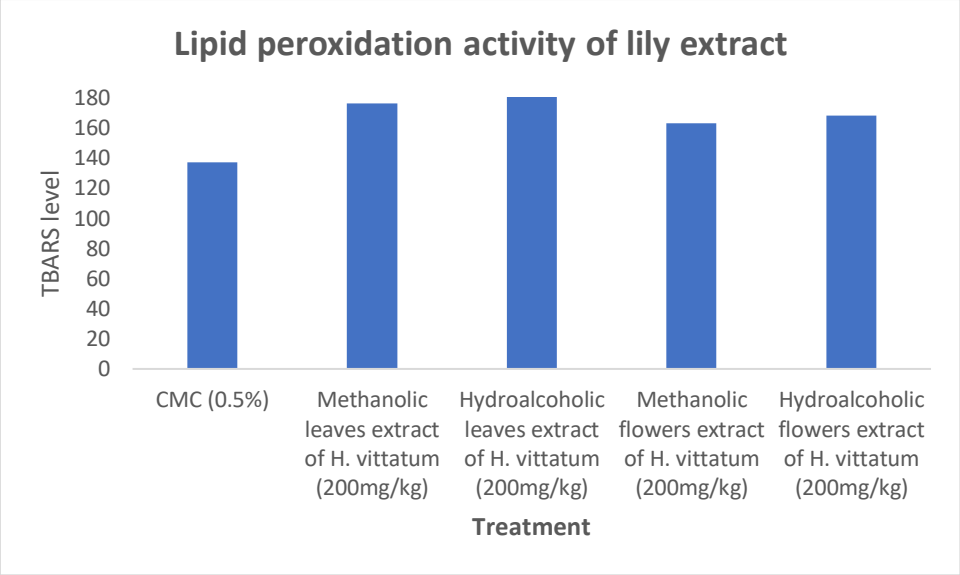


Determination of Lipid peroxidation

TBARS level was estimated as 137.24±1.67 in CMC (0.05%) treated group. Methanolic flowers extract of *H. vittatum* (200mg/kg) and hydroalcoholic flowers extract of *H. vittatum* (200mg/kg) exhibited the TBARS level as 163.26±1.17 and 168.45±1.12, respectively.

Table 4. Lipid peroxidation of lily extract

Treatment	TBARS level
CMC (0.5%)	137.24±1.67
Methanolic leaves extract of *H. vittatum* (200mg/kg)	176.54±1.31
Hydroalcoholic leaves extract of *H. vittatum* (200mg/kg)	183.30±2.34
Methanolic flowers extract of *H. vittatum* (200mg/kg)	163.26±1.17
Hydroalcoholic flowers extract of *H. vittatum* (200mg/kg)	168.45±1.12

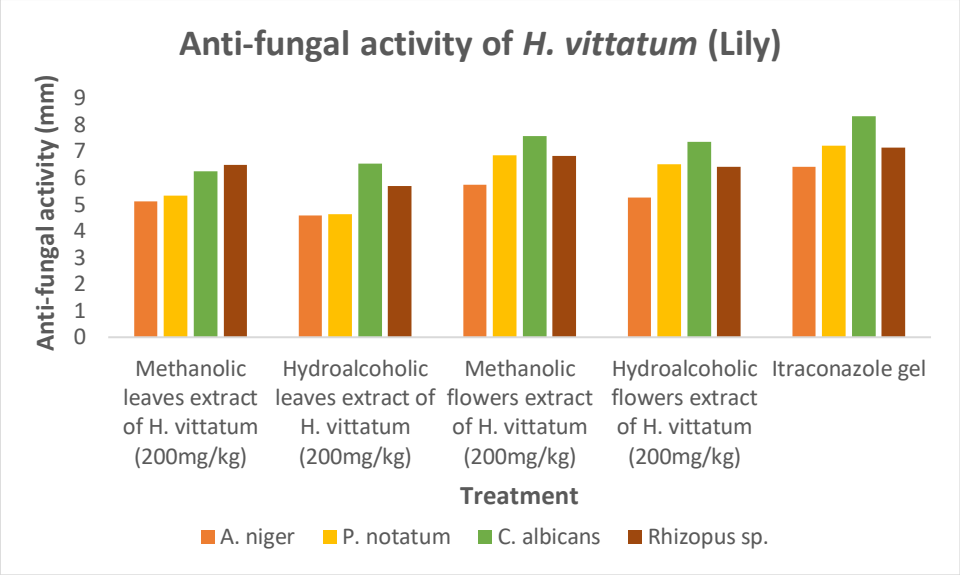


Evaluation of anti-fungal activity

Methanolic leaves extract of *H. vittatum* (200mg/kg) showed the moderate anti-fungal potential (disc diffusion) against all the fungal strains used i.e., *A. niger* (5.10 mm), *P. notatum* (5.32 mm), *C. albicans* (6.24 mm) and *Rhizopus sp.* (6.49 mm). Methanolic flowers extract of *H. vittatum* (200mg/kg) exhibited highest anti-fungal potential as *A. niger* (5.73 mm), *P. notatum* (6.84 mm), *C. albicans* (7.56 mm) and *Rhizopus sp.* (6.81 mm) which was near to standard (Itraconazole gel).

Table 5 1Anti-fungal activity of H. vittatum (Lily)

Formulation	**Anti-fungal activity (mm)**			
	A. niger	***P. notatum***	***C. albicans***	***Rhizopus sp.***
Methanolic leaves extract of *H. vittatum* (200mg/kg)	5.10	5.32	6.24	6.49
Hydroalcoholic leaves extract of *H. vittatum* (200mg/kg)	4.59	4.64	6.53	5.68
Methanolic flowers extract of *H. vittatum* (200mg/kg)	5.73	6.84	7.56	6.81
Hydroalcoholic flowers extract of *H. vittatum* (200mg/kg)	5.26	6.52	7.34	6.40
Itraconazole gel	6.42	7.20	8.31	7.14



Potential possibilities for the search for biologically significant substances include plants, and the findings of our investigation demonstrate the pharmacological characteristics of L. cylindrica. L. cylindrica leaf extracts in ethanol and ethyl acetate shown antibacterial, anti-inflammatory, and antioxidant properties [12]. Throughout the plant kingdom, flavonoids are extensively distributed and frequently found in fruits, vegetables, and some drinks. The plant flavones luteolin (3',4',5,7-tetrahydroxyflavone) and apigenin (4',5,7-trihydroxyflavone) are found in large quantities in a variety of fruits, vegetables, and medicinal plants. It has been discovered that these physiologically necessary flavonoids can be effective as medications [13].

Medicinal plants have been a very important source of medicinal products for millennia. A significant number of drugs used in the medicine of the 21st century have been developed from vegetal sources. Traditionally, plants have been used for their anti-inflammatory, antioxidant, and antibacterial effects. Identifying, testing, and implementing the use of new molecules in current therapeutic protocols is a very complex, time-consuming, and expensive process. In many situations, attention is directed towards the study of plant extracts as it is very well known that, in some cases, the activity of the phytocomplex is superior to that of pure phytochemicals due to the synergism of molecular structures [14].

The research employed the DPPH method, which relies on the color change of DPPH resulting from the reaction between the DPPH free radical and an electron or hydrogen atom released by a compound present in the material, yielding a yellow 1,1-diphenyl-2-picrylhydrazyl compound. The ethanol extract of tamarillo peel was assessed using the DPPH technique, with vitamin C serving as a reference. Vitamin C can be employed as a comparator in this investigation due to its high natural antioxidant properties [15]. This research shown that the ethanol extract of tamarillo peel exhibits strong antioxidant activity. This study parallels the research conducted by Hawa et al., which asserts that diverse kinds have strong antioxidant capacity [16]. The extraction of tamarillo fruit and skin utilizing various solvents, including 80% methanol and aqueous solutions, was assessed by the DPPH, FRAP, and ABTS test methods. Nallakurumban et al. demonstrated that tamarillo contains a substantial quantity of phenolics and flavonoids, which enhance the fruit's antioxidant potential [17].

CONCLUSION

Development of ethanolic *H. vittatum* flowers extract might be very significant in terms of treating cellular oxidation and fungal infections. It can counter the skin problems specially dermatitis, pruritic and other epidermal ailments.

In conclusion, leaves extract of lily showed a moderate DPPH scavenging potential while flowers extract of *H. vittatum* (lily) exhibited a more significant DPPH scavenging potential which might be due to release of more phytochemical constituents in flowers extract. SOD and TBARS level were found modulated significantly by the flowers extract of *H. vittatum* (lily) in contrast to leaves extract of *H. vittatum*. Moreover, methanolic fractions reported a higher level of total phenolic and total flavonoids contents than hydroalcoholic fractions which might be due to better solubility of phytocomponents. Flowers extract of *H. vittatum* (200mg/kg) exhibited highest anti-fungal potential as *A. niger* which was near to standard anti-fungal (Itraconazole gel).

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