



# Peritoneal Mast Cell Stabilization and Free radical Scavenging activity of *Yucca gloriosa* L

Saurabh Gupta<sup>1\*</sup>, Satish Kumar Muthureddy Nataraj<sup>2</sup>, K. Rama Satyanarayana Raju<sup>2</sup>, Shashank Mulukutla<sup>2</sup>, Nilesh Ambore<sup>2</sup> and Renu Gupta<sup>3</sup>

<sup>1</sup>Department of Pharmacology, Indore institute of Pharmacy Pithampur road, Opp. IIM, Rau, Indore (M.P.) 453331, India.

<sup>2</sup>Department of Pharmacology, J.S.S. College of Pharmacy, Off campus J.S.S University, Ootacamund-643 001, Tamil Nadu, India.

<sup>3</sup>Dr. Batra's Homeopathic Clinic, M.G. Road, Indore 452001, Madhya Pradesh, India.

## ABSTRACT

**Objective:** To investigate *in vitro* antioxidant and mast cell stabilization potential *Yucca gloriosa* L. **Methods:** The aerial parts of *Yucca gloriosa* L. (Family-Agavaceae) were successively extracted with ethanol, 50% aqueous-ethanolic and aqueous to prepare an extract of the plant. Preliminary phytochemical tests, total phenol and flavonoid estimation were analyzed in *Y. gloriosa* L. extract. Furthermore, *in vitro* antioxidant activity was evaluated such as DPPH method, ABTS assay, superoxide assay, nitric oxide assay and lipid peroxidation inhibition assay. The extract of YGE, 50% YGE and YGA (1, 10 and 100 µg/ml) was studied for peritoneal mast cell stabilization activity in rat mesenteric preparation induced by C 48/80. **Results:** The result revealed that YGE showed highest concentration of phenol and flavonoid compounds. Free radical scavenger activity and mast cell activity had been identified in YGE, 50% YGE and YGA extracts. The Free radical scavenger study revealed that the YGE, 50% YGE and YGA extracts of *Y. gloriosa* L. showed significant activity in DPPH method, Nitric oxide assay, ABTS cation decolorization assay, superoxide assay and lipid peroxidase assay. All the extracts showed ability to prevent oxidation when compare with standard markers. Upon further investigation for mast cell activity, of YGE extracts showed significant increase the number of intact cells when compared with C 48/80 at the concentrations of 10 and 100 µg/mg when compare to other extracts. **Conclusion:** This finding provides suggested that *Y. gloriosa* L. is a potential candidate in herbal medicine for allergic asthma. Due to its ability to inhibit mast cell derived immediate-type I allergic reactions. It virtues further work towards the isolation of phytoconstituents from *Y. gloriosa* L.

**Key words:** Compound 48/80, Free radicals, Mast cell, *Yucca gloriosa* L.

## INTRODUCTION

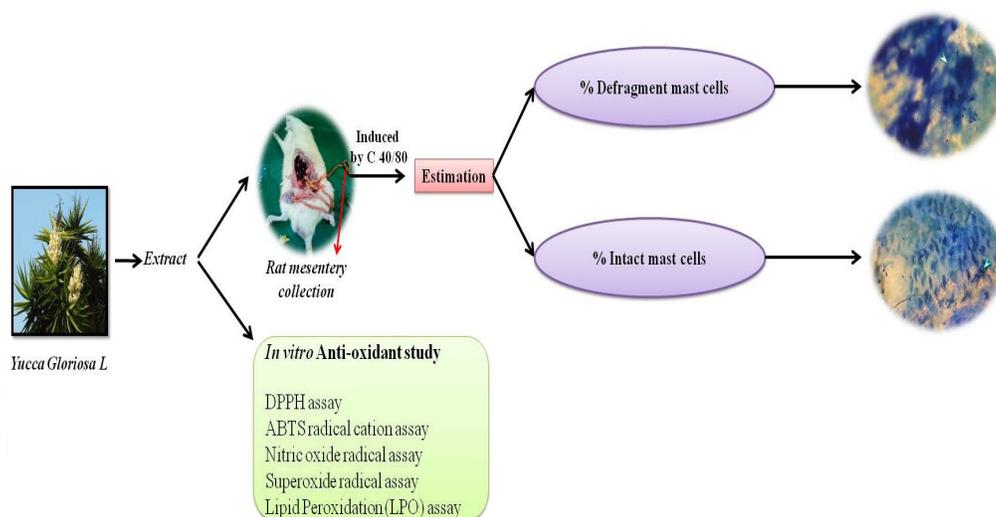
Free radicals and related species have attracted a great deal of attention in recent years. These are mainly derived from oxygen (reactive oxygen species/ROS) and nitrogen (reactive nitrogen species/RNS), and are generated in our body by various endogenous systems, exposure to different physicochemical conditions or

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### \*Address for correspondence:

Dr. Saurabh Gupta, Associate Professor and Head, Department of Pharmacology, Indore Institute of Pharmacy Pithampur road, Opp. IIM, Rau, Indore (M.P.) 453331, India. E-mail: saurabhgupta80@gmail.com



Graphical Abstract

pathophysiological states. Free radical theory has greatly stimulated interest in the role of dietary antioxidants in preventing many human diseases, including asthma, cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration and diabetes. Dietary antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. These effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.<sup>1</sup>

Mast cells are found in skin and all mucosal tissues at homeostasis, and numbers are elevated in asthmatics lungs and inflammatory bowel disease. Mast cells were first described by Ehrlich in his 1878 doctoral thesis on the basis of their unique staining characteristics and large granules, that gave them their name, “Mastzellen” which means well-fed cells, because their cytoplasm was stuffed with granular material. Mast cells are now considered to be part of the immune system.<sup>2</sup> The mast cell was identified as a mesenchymal cell which is stained metachromatically with O-toluidine blue. Several years later; it was recognized that these cells contained in their granules, the majority of the body’s histamine. Mast cells play a central role in inflammatory and immediate allergic reactions. The release of potent inflammatory mediators, such as histamine, proteases, chemotactic factors, cytokines and metabolites of arachidonic acid that act on the vasculature, smooth muscle, connective tissue, mucous glands and inflammatory cells. Histamine is not only released when the body encounters a toxic substance, but also when mast cells detect injury.<sup>3</sup>

*Y. gloriosa* L. (family- *Agavaceae*), known as Spanish Dagger. It is a stem less plant or a rising of small trees and having a short trunk. The leaves of this plant are 2-3 feet long, 2 inches wide, long pointed, often toothed margin, mostly rosettes at the ground level. Flowers have many cups or saucer shaped, hanging, greenish white to reddish, fragrant, born mostly in erect panicles that usually overtop the leaves.<sup>4</sup> *Y. gloriosa* L. widely grow in California, Mexico and India. In India it is widely available in Tamilnadu, Andhra Pradesh and Himalayas. An ethnobotanical survey carried out in ancient times the whole plant was used for bronchitis, asthma, dysentery, phthisis, menstrual disorder, hemorrhagic, septicemia,<sup>5,4</sup> anti-inflammatory agents, arthritis,<sup>6</sup> cancer,<sup>7</sup> cerebral ischemia, hepatoprotective, leprosy, ulcer, anti-helminthic, anti-microbial<sup>8</sup> etc. The fruit is used as a blood purifier, cholagogue and as a purgative. The rhizomes are used in the manufacture of detergent.<sup>5</sup>

The previous literature well explore the phytochemical constituents of *Y. gloriosa* L. leaves contain sapogenins-tigogenin, smilagenin, 12- $\beta$ -hydroxysmilagenin and  $\beta$ -sitosterol, tigogenin 3-O- $\beta$ -D-xylopyranosyl- $\beta$ -lycotetraoside, gitogenin 3-O- $\beta$ -D-xylopyranosyl- $\beta$ -lycotetraoside, gitogenin 3-O- $\alpha$ -L-rhamnopyranosyl- $\beta$ -lycotetraosid, pro-type of gitogenin 3-O- $\beta$ -D-xylopyranosyl- $\beta$ -lycotetraoside,<sup>4,5,9</sup> sitosterol, stigmaterol, campesterol and cholesterol.<sup>5,10</sup> Roots and bark of *Y. gloriosa* L. are a rich source of phenolic compounds like yuccaols (A-E) and gloriosaols (A-E).<sup>11</sup> Flowers of *Y. gloriosa* L. contain yuccaloesides A, B, C, E, degalactotigonin and gitogenin 3-O- $\alpha$ -L-rhamnopyranosyl- $\beta$ -lycotetraosidesteroidal glycosides.<sup>12</sup> The phytochemistry of *Y. gloriosa* L. has been well developed and demonstrated in various literatures. Their rich structural diversity and complexity has prompted

us to further intensify investigation. The present study was undertaken to evaluate the antioxidant and mast cell stabilization property of plant extracts of the aerial part of *Y. gloriosa* L. on rat mesentery, which is not reported so far.

## MATERIAL AND METHODS

### Collection

*Y. gloriosa* L. aerial parts were collected in the month of August, 2010, from Tirupati district, Andhra Pradesh, India. Dr. K. Madhava Chetty, Botanist, Department of Botany, Sri Venkateswara University, Tirupati authenticated the collected plant. Voucher specimen has been preserved in our laboratory (SVU/SC/08/24/10-11) for future reference.

### Chemicals

2,2-Diphenyl-2-picryl hydrazyl (DPPH), 2,2-azinobis(3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS), nitro blue tetrazolium (NBT), butylated hydroxy anisole (BHA), Rutin and *p*-nitroso dimethyl aniline (*p*-NDA) were obtained from Acros Organics, NJ, USA. Naphthyl ethylene diamine dihydrochloride (NEDD) was obtained from Roch-Light Ltd., Suffolk, UK. Sodium nitroprusside, Ascorbic acid, Xylene, Sulphanilic acids, Ethanol, NaCl, KCl, CaCl<sub>2</sub>, NaHCO<sub>3</sub>, Dextrose, Xylene, Acetone and Anesthetic ether were purchased from E-Merck (India) Ltd., Mumbai, India. Thiobarbituric acid (TBA), phenazin methosulfate (PMS), Trichloroacetic acid (TCA), Griess reagent, Quercetin, Gallic acid, Catechin, Folin-Ciocalteu reagent, Compound C40/80 and O-toluidine blue were purchased from Sigma-Aldrich Pvt. Ltd. All chemicals used were of analytical grade. Standard drug Disodium cromoglylate (DSCG) was obtained from Cipla Pvt. Ltd. as a gift sample.

### Extraction procedure

Aerial parts of *Y. gloriosa* L. were cleaned thoroughly with water to remove any unwanted matter, dried in shade, ground to a coarse powder with a mechanical grinder and passed through sieve no. 40. Further, it was extracted with cold maceration process using absolute ethanol by intermittent shaking for 10 days, filtered and the dried. The marc was macerated with 50% water-ethanol mixture (1:1) for 10 days, filtered and again the resultant residue was macerated with water for another 10 days with intermittent shaking. The solvent was removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash

evaporator (Rota vapor, R-210/215, Buchi, Switzerland). The concentrated semi solid material was kept in a desiccator for drying. The extracts of plant, were stored in a refrigerator at 40°C until further use. Henceforth the extracts will be called as YGE, 50% YGE and YGA, respectively.

### Qualitative phytochemical screening

The *Y. gloriosa* L. extracts were subjected to phytochemical tests to find the presence of major phytochemical constituents such as carbohydrates, proteins, amino acids, alkaloids, glycosides, saponins, sterols, flavonoids, phenolic compounds, fixed oil and fat, gum and mucilage, phytosterols and tannins according to standard methods.<sup>13</sup>

### Estimation of total phenolic content

The total phenolic content in the extracts was determined with the Folin-Ciocalteu reagent. 10mg of test extract was dissolved in 10 ml of methanol (1 mg/ml). From this, 400 µl was separately mixed with 2 ml of Folin-Ciocalteu reagent and 1.6 ml of sodium carbonate. After shaking, the mixture was kept for 2 h reaction time. The absorbance was measured at 750 nm (Shimadzu UV-160 A Spectrophotometer, Shimadzu Corporation, Japan). Using Gallic acid monohydrate, a standard curve was prepared and linearity was obtained in the range of 7.84 - 1000 µg/ml. The total phenolic content of tested extracts was calculated by interpolation on the standard curve. All determination was performed in triplicate. The total phenol content was expressed as gallic acid equivalent in mg/g or % w/w of the extracts.<sup>14</sup>

### Estimation of total flavonoid content

Total flavonoid content was measured by aluminum chloride colorimeter assay. 10 mg of tested extracts was dissolved in 10 ml of methanol to get 1 mg/ml solution. From that, 0.5 ml of the test extract was mixed with 1.5 ml methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. Using rutin, standard curve was prepared and linearity was obtained in the range of 10-100 µg/ml. The total flavonoid content of tested extracts was determined by interpolation of unknown absorbance on the standard curve. All determination was performed in triplicate. The total flavonol content was expressed as rutin equivalent in mg/g or % w/w of the extract.<sup>14</sup>

## **In vitro free radical scavenging activity**

### *DPPH radical scavenging activity*

DPPH radical scavenging activity was measured by spectrophotometric method. To an ethanolic DPPH solution (200  $\mu$ M), 1mg of test extract dissolved in ethanol was added at different concentration (31.25-1000  $\mu$ g/ml). The mixture was incubated at 37°C for 20 min in dark and absorbance of each solution was determined at 517 nm. Synthetic antioxidant ascorbic acid was used as positive control.<sup>15</sup> Absorbance was measured against a blank solution containing the extract or standard, but without the reagents. A control test was performed without the extracts or standards. Percentage scavenging and IC<sub>50</sub> values  $\pm$  S.E.M. (IC<sub>50</sub> value is the concentration of the sample required to inhibit 50% of radical) were calculated.

### *Nitric oxide scavenging activity*

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside (5 mM) in standard phosphate buffer solution (0.238 g of disodium hydrogen phosphate and 0.019g of potassium dihydrogen phosphate and 0.8g of NaCl in 100 ml distilled water adjust pH 7.4) was incubated. 1mg of test extract was dissolved in ethanol and was added at different concentration (31.25-1000  $\mu$ g/ml) to phosphate buffer (0.025 M; pH: 7.4) and the tubes were incubated at 25°C for 5 h. Positive control experiments, without the test compounds but with equivalent amounts of buffer were conducted in an identical manner. After 5 h, 0.5 ml of incubated solution was removed and diluted with 0.5 ml of griess reagent (1% sulpanilamide, 2% *o*-phosphoric acid and 1% naphthyl ethylene diamine dihydrochloride). The absorbance was measured at 546 nm. Synthetic antioxidant, ascorbic acid was used as positive control.<sup>15</sup>

### *ABTS radical scavenging activity*

ABTS radical cations were produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulfate. The mixture was allowed to stand in the dark at room temperature for 12-16 h before use. 1mg of test extract dissolved in ethanol and was added at different concentrations (31.25-1000  $\mu$ g/ml) of the extracts (0.5 ml) was added to 0.3 ml of ABTS solution and the final volume was made up to 1 ml with ethanol and incubated for 20 min. Absorbance of these solutions were measured spectrophotometrically at 734 nm. Synthetic antioxidant ascorbic acid was used as positive control.<sup>15</sup>

### *Superoxide radical scavenging assay*

The superoxide anion radical scavenging activity was assayed. Superoxide radicals were generated in 3.0 ml of

Tris-HCl buffer (16 mM, pH 8.0), which contained 78  $\mu$ M  $\beta$ -nicotinamide adenine dinucleotide (reduced form NADH), 50  $\mu$ M nitroblue tetrazolium (NBT), 10  $\mu$ M phenazin methosulfate (PMS), and 1mg of test extract dissolved in 50% ethanol, which was added at different concentrations (31.25-1000  $\mu$ g/ml). The color reaction of superoxide radicals and NBT absorbance was measured at 560 nm. Synthetic antioxidant (+)-catechin was used as positive controls.<sup>16</sup>

### *Lipid peroxidation inhibition assay*

Lipid peroxidation inhibitory activity of three extracts, the compound and standard were carried out according to the method. Egg lecithin (3 mg/ml, phosphate buffer, pH 7.4) was sonicated. The test extracts (100  $\mu$ l) at different concentrations (31.25-1000  $\mu$ g/ml) were added 1mL of liposome mixture, control was without test sample. Lipid peroxidation was initiated by adding 10  $\mu$ l FeCl<sub>3</sub> (400 mM) and 10  $\mu$ l of L-ascorbic acid (200 mM). After incubation for 1 hour at 37°C, the reaction was stopped by adding 2 ml of 0.25 N HCl containing 15% trichloro acetic acid (TCA) and 0.375% thiobarbituric acid (TBA). The reaction mixture was boiled for 15 min, cooled, centrifuged and the absorbance of the supernatant was measured at 532 nm. Synthetic antioxidants ascorbic acid was used as positive control.<sup>17</sup>

### *Animals*

Healthy male albino Wistar rats (180–220 g) were obtained from the Animal House, J.S.S. College of Pharmacy, Ootacamund, India, and were maintained under standard environmental conditions (22–28°C, 60-70% relative humidity, 12-h dark:12-h light cycle). They were fed with standard rat feed (M/S Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (Approval no. JSSCP/IAEC/Ph.D/P.Cog/02/2011-12).

### *Mast cell stabilization activity*

The animals were fasted overnight and sacrificed with excess dose of anesthetic ether. The abdomen was cut open to expose the intestine. Pieces of mesentery with connecting lobes of fat and blood vessels were rapidly dissected out. A small pieces of the mesentery were cut and placed in a beaker containing Ringer Locke (in mM: NaCl 154, KCl 5.6, CaCl<sub>2</sub> 2.2, NaHCO<sub>3</sub> 6.0 and dextrose 5.5) solution for 30  $\pm$  1 min containing different concentrations of plant extracts. Later, the tissues were exposed to compound C48/80 (C 48/80 at 0.8  $\mu$ g/ml to promote mast cell degranulation) and the tissue was incubated for further 30  $\pm$  1 min. The pieces of mesentery were removed and placed in a clean slide. Excess fatty

layers and adhering small intestine tissues were removed. The trimmed tissue was dipped in 4% formaldehyde solution containing 0.1% O-toluidine blue for 20-30 min and then was transferred through acetone and then xylene (2 changes each) for  $5 \pm 1$  minute. Six pieces of mesentery were used for each concentration of the test substance. The stained mesentery pieces were focused through a digital light microscope (M/s. Motic, Japan) at 100x magnification. 100 mast cells were counted, starting from the left side of the field and then proceeding clockwise. The number of intact and fragmented or disrupted mast cells was noted. A mast cell was considered disrupted if 4 or 5 granules were found around the mast cells. The percentage of mast cell fragmented or disrupted and of intact mast cells was calculated.<sup>18</sup>

The following experimental groups were used for the study

- Group I: Vehicle control (tissues exposed to Ringer Locke solution only)
- Group II: Negative control (tissues exposed to 0.8 µg/ml of C 48/80 only)
- Group III: Positive control (tissues exposed to disodium cromoglylate-DSCG, 1 mg/ml)
- Group IV: Tissues exposed in ethanolic extract of *Y. gloriosa* L. (YGE) 1 µg/ml
- Group V: Tissues exposed in ethanolic extract of *Y. gloriosa* L. (YGE) 10 µg/ml
- Group VI: Tissues exposed in ethanolic extract of *Y. gloriosa* L. (YGE) 100 µg/ml
- Group VII: Tissues exposed in 50% aqueous ethanolic extract of *Y. gloriosa* L. (50% YGE) 1 µg/ml
- Group VIII: Tissues exposed in 50% aqueous ethanolic extract of *Y. gloriosa* L. (50% YGE) 10 µg/ml

- Group VIII: Tissues exposed in 50% aqueous ethanolic extract of *Y. gloriosa* L. (50% YGE) 100 µg/ml
- Group X: Tissues exposed in aqueous extract of *Y. gloriosa* L. (YGA) 1µg/ml
- Group XI: Tissues exposed in aqueous extract of *Y. gloriosa* L. (YGA) 10 µg/ml
- Group XII: Tissues exposed in aqueous extract of *Y. gloriosa* L. (YGA) 100 µg/ml

All the groups except group I and II were later exposed to 0.8 µg/ml of C 48/80.

### Statistical analysis

Statistical analysis was done by using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison tests. *P* value<0.05 was considered to be statistically significant. The analysis was carried out using Graph Pad Prism software V.5.04.

## RESULTS

### Extract recovery percent

The maximum yield was obtained in 50% water-ethanol mixture when compared to other solvents. The extractive values were found to be: 3.16%, 13.29% and 7.43% w/w for ethanolic, 50% aqueous ethanolic and aqueous extracts respectively.

### Qualitative phytochemical screening

The phytochemical screening on *Y. gloriosa* L. revealed the presence of primary metabolites such as carbohydrates, proteins and amino acids. The secondary metabolites found included alkaloids, glycosides, saponins, sterols, flavonoids, phenolic compounds and tannins (Table 1).

**Table 1: Preliminary phytochemical screening of *Yucca gloriosa* L. extracts**

Phytochemicals	Ethanolic	50% ethanolic	Aqueous
Carbohydrates	+++	+++	+++
Proteins	+	-	-
Amino acids	-	-	-
Alkaloids	-	++	+++
Saponins	+++	+++	+++
Phenolic compounds	+++	++	++
Tannins	++	+	+
Flavonoids	++	+++	++
Glycosides	++	+	-
Phytosterols	+++	+++	+++
Fixed oils and fats	-	-	-
Gums and mucilages	-	-	-

(+): Presence of chemical compounds; (-): absence of chemical compounds; (+)<(++)<(+++): Based on the intensity of characteristic colour.

**Table 2: In vitro antioxidant activity of *Y. gloriosa* L. extracts**

Extract/ Compound	*IC <sub>50</sub> Values ± S.E.M. (µg/ml)				
	DPPH	Nitric oxide	ABTS	Super oxide	Lipid peroxidation
YGE	62.4±0.5	68.02±1.3	90.7±1.6	66.6±1.1	164.6±1.4
50% YGE	103.3±0.3	22.2±0.9	138.4±1.5	116.0±0.9	198.0±0.7
YGA	198.2±0.8	290.0±1.1	95.3±2.1	294.9±0.9	286.6±1.02
Standard					
Ascorbic acid	5.5±0.1	47.2±0.4	12.3±0.2	-	147.9±1.5
+/- Catechin	-	-	-	42.03±1.5	-

\*Average Mean±SEM of three values; -Mean no value.

## Quantification of total phenolics and flavonoids

The total phenolic content revealed that the YGE extract (186.6 mg of GAE/g extract) contained high amounts of phenolic compounds. Among the different extracts analyzed, YGE showed better total phenolic content as compared to the others. The total phenolic content of YGE, 50% YGE and YGA was found to be 186.6, 141.4 and 90.8 mg of GAE/g extract, respectively.

Among the extracts analyzed, YGE (32.4 mg of RE/g extract) showed better total flavonoid content as compared to the other two extracts. The total flavonoid content of YGE, 50% YGE and YGA extracts were found to be 32.4, 28.7 and 20.2 mg of RE/g extract, respectively. However, absolute ethanol was found to be a more efficient solvent for extracting the phenolic and flavonoid content from *Y. gloriosa* L.

## In vitro free radical scavenging activity

### DPPH radical scavenging activity

DPPH radical scavenging activities of different extracts of *Y. gloriosa* L. are showed in (Table 2). The lower value of IC<sub>50</sub> indicated a higher antioxidant activity. Among the tested extracts, the ethanolic extract of *Y. gloriosa* L. revealed a better DPPH radical scavenging capability when compared to other extracts. The IC<sub>50</sub> value of YGE was found to be 62.4 ± 0.5 µg/ml, whereas that of 50% YGE and YGA was 103.3 ± 0.3 and 198.2 ± 0.8 µg/ml, respectively. The standard used in DPPH assay was ascorbic acid. The IC<sub>50</sub> value of standard antioxidant was found to be (5.5 ± 0.1 µg/ml).

### Nitric oxide scavenging activity

Nitric oxide scavenging activities of different extracts of *Y. gloriosa* L. are showed in (Table 2). The YGE extract demonstrated better reduction in nitric oxide scavenging activity (68.02 ± 1.3 µg/ml) when compared to that of 50% YGE (122.2 ± 0.8) and YGA extracts (290.0 ± 1.1 µg/ml).

The IC<sub>50</sub> of standard antioxidant was found to be 47.2 ± 0.4 µg/ml. The standard used in the assay was ascorbic acid.

### ABTS radical scavenging activity

ABTS radical scavenging activities of *Y. gloriosa* L. extracts are showed (Table 2). Among all the extracts under investigation, the YGE extract showed better ABTS radical scavenging activities compared to other solvent extracts. The IC<sub>50</sub> of YGE extract was found to be 90.7 ± 1.6 µg/ml while that of 50% YGE and YGA was 138.4 ± 1.5 and 95.3 ± 2.1 µg/ml, respectively. The standard used in ABTS assay was ascorbic acid. The IC<sub>50</sub> of standard antioxidant were found to be (12.3 ± 0.2 µg/ml).

### Superoxide radical scavenging assay

Superoxide anion radical scavenging activities of various extracts of *Y. gloriosa* L. are showed in (Table 2). Among all the extract, the YGE extract showed better superoxide radical scavenging activities compared to other solvent extracts. The IC<sub>50</sub> of YGE extract was found to be 66.6 ± 1.1 µg/ml whereas that of 50% YGE and YGA was 116.0 ± 0.9 and 294.9 ± 0.9 µg/ml, respectively. The standard used in superoxide assay was ± Catechin. The IC<sub>50</sub> of standard antioxidant were found to be (42.0 ± 1.5 µg/ml).

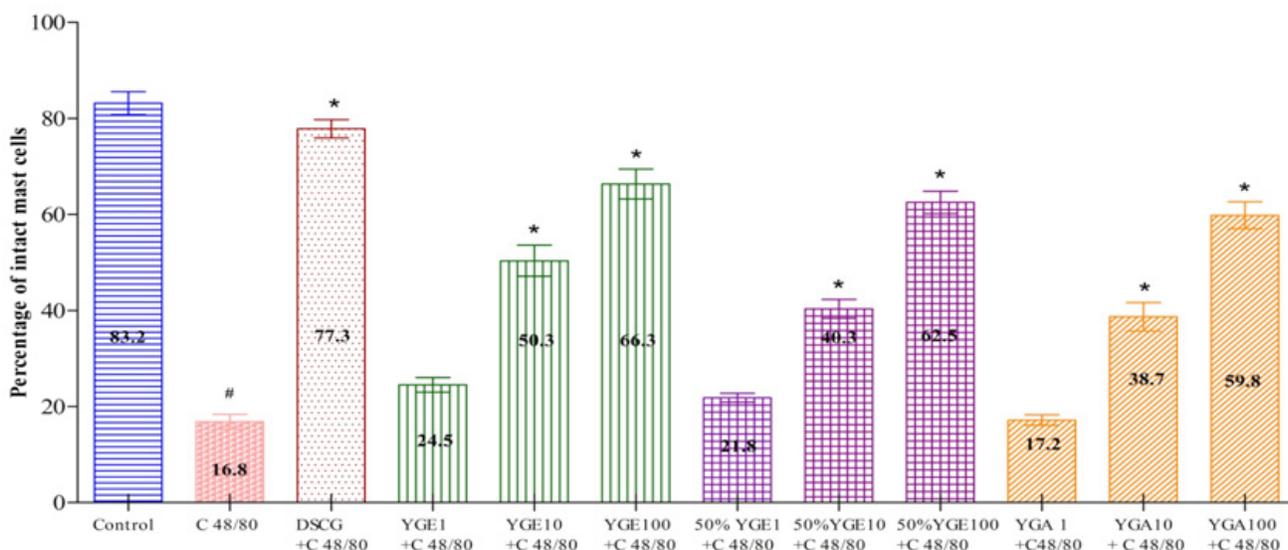
### Lipid peroxidation inhibition assay

Lipid peroxidation inhibition assay of different extracts of *Y. gloriosa* L. are shown (Table 2). Among all the extracts, the YGE extract showed better Lipid peroxidation inhibition assay when compared to other extracts. The IC<sub>50</sub> of YGE extract was found to be 164.6 ± 1.4 µg/ml whereas that of 50% YGE and YGA was 198.0 ± 0.7 and 286.6 ± 1.02 µg/ml, respectively. The IC<sub>50</sub> of standard antioxidant were found to be 147.9 ± 1.59 µg/ml.

## In vivo studies

### Mast cell stabilization activity

Compound 48/80, a known mast cell degranulation agent, produced a significant ( $P < 0.001$ ) reduction in intact



**Figure 1 : Effect of *Y. gloriosa* L. extracts on mast cell degranulation induced by compound 48/80 in the rat mesentery**  
 #  $p < 0.001$  vs. control; \*  $p < 0.001$  vs. C 48/80; One way ANOVA followed by Tukey's multiple comparison test ( $F=107.4$ ;  $df=71$ ;  $p < 0.001$ )  
 DSSG-disodium cromoglycate 1mg/ml, *Y. gloriosa* L. extract (ethanolic-YGE, 50% aqueous ethanolic -50% YGE and aqueous-YGA)

mesenteric mast cells,  $16.8 \pm 1.5$ , when compared to the mesentery exposed to the vehicle, Ringer Locke's solution, alone  $83.2 \pm 2.4$ . At the concentration of 10 and 100  $\mu\text{g}/\text{ml}$ , ethanolic, 50% aqueous ethanolic and aqueous extracts of *Y. gloriosa* L. produced dose dependent and showed significant ( $P < 0.001$ ) increase in the number of intact cells when compared with C48/80 treated tissues (Figure 1). Among the different extracts ethanolic YGE 10  $\mu\text{g}/\text{ml}$  ( $50.3 \pm 3.2$ ) and 100  $\mu\text{g}/\text{ml}$  ( $66.3 \pm 3.1$ ) showed significant protection of intact mast cell as compared other two extract. The mast cell stabilization of different extracts follows the order: YGE > 50% YGE > YGA respectively.

## DISCUSSION

Complementary and alternative medicine has been used to treat asthma for hundreds of years.<sup>19</sup> Indian systems of traditional medicine are well systematized, but are largely unrecognized in the West. Ayurveda is gaining greater visibility; however, related systems, such as Unani-Tibb and Siddha, are more obscure. Ayurvedic drugs having a great interest for consideration in asthma were subjected to clinical studies based on their effects in experimental studies.<sup>20</sup> Due to this consideration, the present study is focused in the direction of healing allergic asthma by herbal medicine.

The qualitative phytochemical investigation of the test extracts proved the presence of carbohydrates, proteins and amino acids in all extracts of *Y. gloriosa* L. which brought

to therefore, it's possible application as a good nutritional supplement, provided it is free from the microbial factors. It is also noted that the other phytochemical compounds detected are known to have a beneficial impact for industrial output. The higher concentrations of saponins, sterols, alkaloids, flavonoids, phenolic compounds and tannins present in all extracts of the plant make it a good source for isolation and purification in the future. Previous literature has justified the medicinal role of saponins, alkaloids and phenolic compounds and their use in hypercholesterolemia, hyperglycemia, as anti-allergic, antioxidants, anticancer agents and anti-inflammatory agents, etc.<sup>21</sup> Phenolic compounds have been known to possess a capacity to scavenge free radicals. They are commonly found in both edible and non-edible plants, and have multiple biological effects, including antioxidant activity.<sup>22</sup> Due to this reason, nowadays, much attention is given towards their antioxidant potential. Therefore, the estimation and characterization of plant extracts of *Y. gloriosa* L. for phenolic and flavonoid content and antioxidant capability should be done to explore the bioactive potential of such phytoconstituents. The present study revealed that *Y. gloriosa* L. is a rich source of phenolic and flavonoid compounds in all the extracts except the ethanolic extract of the plant.

Free radicals or antioxidants can be of two types depending upon its origin, namely, reactive oxygen species (ROS) and reactive nitrogen species (RNS). The antioxidant activity of phenolic compounds is principally due to their redox properties, which allow them to act as reducing agents,

hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelation potential. Phenolic compounds, such as flavonoids, phenolic acids, stilbenes, lignin and tannins, are especially present in different plant parts such as leaves, flower and woody parts such as stems.<sup>23</sup> The result revealed that the ethanolic extract of *Y. gloriosa* L. (YGE) exhibited strong scavenging effect on 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), nitric oxide, superoxide radical and lipid peroxidation assay. The free radical scavenging effect of *Y. gloriosa* L. was comparable with that of reference antioxidants. The total phenol content and flavonoid content showed strong correlation with antioxidant activity for *Y. gloriosa* L. This indicates that the antioxidant activity of the extracts from *Y. gloriosa* L. aerial parts is due to its phenolic constituents. Larson has proposed that antioxidant play an important preventive role in the development of cancer, asthma, heart disease, inflammation, diabetes and ageing-related diseases etc.<sup>24</sup>

Allergies such as asthma, allergic rhino-conjunctivitis, anaphylaxis and atopic dermatitis have been well known as immediate reactions following contact with certain exogenous allergens.<sup>25</sup> An anaphylactic reaction is also caused by the release of inflammatory mediators (like histamine) from mast cells.<sup>26</sup> Mast cells are mononuclear and granule-containing secretory cells. They are important in the development of many physiological changes during allergic and anaphylactic responses. It has been reported that allergies are associated with the T-helper (Th) 2 type immune response, i.e. activation of interleukin (IL)-4 and immunoglobulin (IgE) synthesis.<sup>27</sup> Therefore the stabilization of mast cell activity could be expected to be of therapeutic value in the treatment of asthma. Mast cell stabilizers such as cromolyn sodium, nedocromil sodium and ketotifen have been successfully used as prophylactic agents for the treatment of asthma. It has been reported that the degranulation of mast cells can be elicited by non-immunologic stimulators such as neuropeptides, basic compounds, complement components, and certain drugs.<sup>28</sup>

It is well known that compound 48/80 (a condensation product of N-methyl-p methoxyphenethylamine with formaldehyde) has the potential to secrete allergy-related factors from mast cells as stimulators.<sup>29</sup> The compound 48/80 has been used to study allergies and anaphylaxis, because it can vigorously activate the release of histamine via the mechanism of cellular exocytosis. Several flavonoids have been established to have smooth muscle

relaxant and bronchodilator activity.<sup>30</sup> The phytochemical and quantitative screening on *Y. gloriosa* L. revealed the presence of such as flavonoids and phenolic compounds. Flavonoids also inhibited the histamine release induced by C 48/80.<sup>29</sup> The various phytochemical compounds are present in *Y. gloriosa* L. like yuccaols (A-E) and gloriosaols (A-E)<sup>11</sup> yuccaloesides A, B, C, E, degalactotigonin.<sup>12</sup> Gupta et al investigation findings suggested that *Y. gloriosa* extract and isolated gloriosaol isomeric mixture (G) showed promising activity on Ovalbumin Induced airway hyperresponsiveness in Balb/C Mice. *Y. gloriosa* and GLM showed very promising activity on inflammatory markers.<sup>31</sup> Our findings revealed that *Y. gloriosa* L. stabilizes mast cells in rat mesenteric tissue, as the mast cells play a major role in Type I hypersensitivity-mediated diseases like allergic asthma and rhinitis.<sup>3</sup> Further studies are under way to evaluate the efficacy of *Y. gloriosa* L. due to its mast stabilization property in these animal allergic models. This could be attributed due to the presence of rich chemical constituents in *Y. gloriosa* L. and potential compounds for allergic anti-asthmatic activity, which shall be confirmed by further molecular and mechanistic approach studies.

## CONCLUSION

This finding provides evidence that the *Y. gloriosa* L. rich chemical constitution inhibits mast cell derived immediate type-I allergic reactions and mast cell degranulation. *Y. gloriosa* will be the potential candidate for allergic anti-asthmatic activity. Studies are underway to evaluate the efficacy of *Y. gloriosa* due to its mast stabilization property in these animal allergic models. It virtues further work towards the isolation of phytoconstituents from this plant and evaluate the study on different animal models with molecular approach towards the pro-inflammatory markers.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

**ABBREVIATION**

<i>Y. gloriosa</i> :	<i>Yucca Gloriosa</i>	BHA:	Butylated hydroxy anisole
YGE:	<i>Yucca gloriosa</i> ethanolic extract	p-NDA:	p-nitroso dimethyl aniline
50% YGE:	50% <i>Yucca gloriosa</i> ethanolic extract	NEDD:	Naphthyl ethylene diamine dihydrochloride
YGA:	<i>Yucca gloriosa</i> aqueous extract	NaCl:	Sodium hydroxide
ROS:	Reactive oxygen species	KCL:	Potassium chloride
RNS:	Reactive nitrogen species	NaHCO <sub>3</sub> :	Sodium bicarbonate
DPPH:	2,2-Diphenyl-2-picryl hydrazyl	TBA:	Thiobarbituric acid
ABTS:	2,2-azinobis(3- ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt	PMS:	Phenazin methosulfate
NBT:	Nitro blue tetrazolium	TCA:	Trichloroacetic acid
		Compound C40/80:	N-methyl-p methoxyphenethylamine
		DSCG:	Disodium cromoglylate
		IC <sub>50</sub> :	Inhibitory concentration a 50%

**Highlights of Paper**

- Research paper is focused to find out an alternative herbal medicine for the allergic rhinitis and asthmatic disorder.
- As mast cell is one of the leading initiator component of type I hypersensitivity allergic reaction.
- So in this direction we assess *Yucca gloriosa* activity on peritoneal mast cell stabilization activity on rat mesenteric preparation.

**Author Profile**

- **Dr. Saurabh Gupta:** M. Pharma., Ph.D. is presently working as an Associate Professor and Head, Department of Pharmacology at Indore institute of Pharmacy, Indore, Madhya Pradesh. My current area of research is natural product, Asthmatic pharmacology, Cellular and molecular biology. He is serving as an editorial member of several reputed journals like Austin Journal of Pharmacology and Therapeutics, World Journal of Pharmaceutical Research and WJPPS & expert Reviewers for Journals like International Immunopharmacology, Journal of Clinical Pharmacology and Biopharmaceutics and Journal of Physiology and Pharmacology and many more. I have authored 15 research articles and one book. I am a life member of Indian pharmaceutical society.



- **Late Dr. Satish Kumar Muthureddy Nataraj:** M. Pharma., Ph.D. had worked as an Professor and Head, Department of Pharmacology at JSS College of Pharmacy, Rockland's, Ooty, Tamilnadu. Expertise in research area of natural product, Cancer, Neurodegenerative disorder, Asthmatic pharmacology, Cellular and molecular biology. He was serving as an editorial member of several reputed journals. He had authored more the 34 research articles in national and international journals. He had presented 31 national research presentation and 15 international research presentation.

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