Phytochemical and Pharmacological evaluation of hibiscus *Hibiscus hispidissimus* griff

Alekhya V<sup>1,2</sup>, Ganapaty S<sup>1</sup>, Deepan T<sup>2</sup>

<sup>1</sup>Department of Pharmacognosy, GITAM Institute of Pharmacy, Gandhinagar, Rushikonda, Visakhapatnam-530045, Andhra Pradesh, India

<sup>2</sup>Department of Pharmacognosy, GIET School of Pharmacy, NH-16, Chaitanya knowledge city, Rajamahendravaram-533296, Andhra Pradesh, India

**Article History:**
- Received on: 08 Sep 2020
- Revised on: 08 Oct 2020
- Accepted on: 10 Oct 2020

**Keywords:**
Anti-bacterial, Antifungal, Anti-inflammatory, Phytochemical studies

**ABSTRACT**

To assess phytochemical with pharmacological studies of *Hibiscus hispidissimus* griffbelong to family malavaceae. Preliminary phytochemical analysis reveals the presence of steroids, triterpenes, saponins, steroidal saponins and phenols. Evaluation of anti-inflammatory, anti-microbial with antioxidant action were performed on aerial parts of methanolic extract of *Hibiscus hispidissimus*. Invitro antioxidant activity was performed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay, hydroxy radical scavenging method and superoxide radical scavenging activity. The results of invitro antioxidant study reveal that % inhibition of *H. hispidissimus* was higher compared to ascorbic acid. Anti-inflammatory studies were performed using carrageenan-induced rat paw oedema animal model, for anti-inflammatory studies, the extracts were compared with standards like indomethacin, and it shows a remarkable zone of inhibition ranging from 58.97 to 71.73 respectively. The antibacterial and antifungal activity of plant extracts were studied for the occurrence of inhibition zones. The activity was performed by the cup plate method. Ethanolic extract of *H. Hispidissimus* shows significant anti-bacterial effect against *S. Aureus, B. Subtilis, P. Vulgaris* and *E. coli* using ciprofloxacin (50 g/ml) as standard. The extracts show remarkable inhibition of zone of inhibition, and results were compared with that of standard drugs against the organism tested. In conclusion, the ethanolic extract of *H. hispidissimus* shows significant antioxidant, anti-inflammatory and anti-bacterial properties.

**INTRODUCTION**

Plants have been used for medicinal purpose long before prehistoric period. Traditional system of medicine continues to be widely precised on many accounts. Drugs obtained from natural sources are used as a reservoir for many biochemical products which are used as extractions for development of many formulations which are non-reactive, nontoxic and free from side effects. Drug resistance for transmittable ailments have directed to enlarged importance in the use of natural product as medicine for diverse human illnesses (Das, 2016). *Hibiscus hispidissimus* Griffith (synonym Hibiscus furcatus DC. non wild., Hibiscus aculcatus Roxb.non walter), locally as ‘coffort root’ or ‘Big thicket Hibiscus’ or ‘Pine Hibiscus’ in English and “Uppancham” in Malayalam. Has another name wild hibiscus (*Hibiscus hispidissimus*, 1854) belong to the family malavaceae shown in Figure 1 (India.
The plant is widely distributed in southwestern parts of India, South Africa, Sri Lanka, Myanmar and Thailand (Harborne, 1994).

Hibiscus species are medicinally important and many of the species are evaluated for their antioxidant and antibacterial properties. The selected species had greater tribal importance as medicine for many human ailments like digestion, as anthelminthic, cooling drink, remedy for poisons, swellings and for eye disorders and also in treating liver disorders especially in southern parts of India. Hence, the present work was to investigate phytochemical and pharmacological activity for Hibiscus hispidissimus.

**Figure 1: Hibiscus hispidissimus**

**MATERIALS AND METHODS**

**Collection of plant material**

Plants of *H. hispidissimus* were collected from west Godavari, Andhra Pradesh. The plant was washed, collected and dried at room temperature. Then ground to a coarse powder, macerated with ethanol and the extract was dried by rotary vacuum. The dried residue was preserved in an airtight container and kept at 4-5°C until further use.

**Phytochemical screening**

The extracts of *H. hispidissimus* were subjected for the presence of phytochemical constituents (Jeffrey, 1973; Sofawara, 1996; Evans, 2002) such as steroids, terpenoids, phenolic compounds, flavonoids, saponins, alkaloids, glycosides, iridoids and protein.

**Antioxidant activity**

Antioxidant assay of *H. hispidissimus* was performed by using three methods such as DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) radical scavenging activity, Hydroxyl radical scavenging activity, Superoxide radical scavenging activity (Khan et al., 2014; Brand-Williams et al., 1995; Fijesh et al., 2010).

**DPPH assay**

The antioxidant potential was assessed by measuring free radical. DPPH dissolved in methanol was prepared, 1ml of this solution was added to 3ml of test, standard and control solution at different concentration (5, 10, & 50 μg/ml).

The absorbance of resulting solutions was measured at 517 nm using UV-visible spectrophotometer. The DPPH radical scavenging was calculated by using the following formula.

\[
\text{DPPH radical scavenging activity} = \frac{\text{Abs(control)} - \text{Abs(Test)}}{\text{Abs(control)}} \times 100
\]

**Hydroxyl radical scavenging activity**

To measure the competition between deoxyribose and test compounds for hydroxy radicals generated, hydroxy radical scavenging activity was used. Extracts of different concentrations ranging from (0.1–1000 μg/ml) were added to the reaction mixture contains 1 ml of potassium phosphate buffer (10 mM, pH 7.4).

The time of incubation was 1 hr at 37°C, then added every 1 ml of 1% thiobarbituric acid and 2.8% of TCA. The resulting mixture was subjected to under-water heating bath for 15 mts. The resulting solution was measured at 532 nm after cooling.

\[
\text{Hydroxy radical scavenging activity} = \frac{\text{Abs(control)} - \text{Abs(Test)}}{\text{Abs(control)}} \times 100
\]

**Superoxide radical scavenging assay**

A sample extract of varying concentration [5-50 μg/ml] test and standard were prepared. The reaction mixture contains (phosphate buffer pH 7.4), 100μl of riboflavin solution (20 μg), 200μl of EDTA (12mμl), 100 μl of NBT (0.1 mg), 1ml of NADH diluted with 3 ml phosphate buffer. The resulting solution was measured at 560 nm. The outcomes are revealed as percentage inhibition.

\[
\text{Superoxide assay} = \frac{\text{Abs(control)} - \text{Abs(Test)}}{\text{Abs(control)}} \times 100
\]

**Anti-microbial study**

The extract of *H. hispidissimus* extracts was investigated against four pathogenic microorganisms, *Bacillus subtiliss*, *Staphylococcus sp*, *Escherichia coli*, *Proteus Vulgaris* by cup plate method (Khan et al., 2013; Dabur et al., 2008).

Test and standard were prepared, agar medium was prepared in a cup with sterile borer, then added 0.05 ml of test solution by using a micropipette. Ciprofloxacin a potent antibiotic (50 μg/dl) was used as standard. All glass plates were incubated for 24 h at 37°C. The diameter of the inhibition zone was recorded.
Anti-inflammatory activity

Anti-inflammatory studies were performed using carrageenan-induced rat paw oedema animal model. Samples extract was administered orally (100 & 250 mg/kg) body weight of each extract and standard (Indomethacin 20mg/kg) by rat paw oedema. Indomethacin (20 mg/kg) used as standard. Oedema developed was measured by volume displacement method (Bhatt et al., 1977; Hafeez et al., 2013).

Outcomes were stated as the percentage of inhibition of oedema calculated by using formula (1-\(\frac{V_t}{V_C}\)) × 100, Where \(V_t\) and \(V_C\) are mean paw volume in treated and control group, respectively. Results were analysed using one-way ANOVA method.

Table 1: Phytochemical analysis of \(H.\) hispidissimus

| Sl.no | Name of the test | Methanolic extract of \(H.\) hispidissimus |
|-------|------------------|------------------------------------------|
| 1     | Steroids         | +                                        |
| 2     | Triterpenes      | +                                        |
| 3     | Saponins         | +                                        |
| 4     | Steroidal Saponin| +                                        |
| 5     | Glycosides       | -                                        |
| 6     | Alkaloids        | -                                        |
| 7     | Carbohydrates    | -                                        |
| 8     | Flavonoids       | +                                        |
| 9     | Tannins          | -                                        |
| 10    | Phenols          | +                                        |
| 11    | Irioids          | -                                        |
| 12    | Cardiac Glycosides| -                                |
| 13    | Mucilage         | +                                        |
| 14    | Proteins & Amino Acid | -                        |

+ve: Present, – ve: Absent

RESULTS AND DISCUSSION

Phytochemical analysis

The phytoconstituents of \(H.\) hispidissimus showed the occurrence of steroids, triterpenes, saponins, flavonoids, mucilage and phenol. The composition of Phytoconstituents in the plant has been mentioned in Table 1.

Antioxidant activity

Three in-vitro antioxidant methods were used such as DPPH assay, hydroxyl radical assay and superoxide radical assay. Antioxidant activity results were expressed in terms of IC\(_{50}\) values. The IC\(_{50}\) values were calculated using the DPPH method for \(H.\) hispidissimus, and ascorbic acid was 42 & 12 µg/ml. The results are expressed in Table 2 and Figure 2. The IC\(_{50}\) values using hydroxy radical scavenging method are 2.8 & 1 µg/ml for ascorbic acid. Results are expressed in Figure 3. For the superoxide radical scavenging assay method, the extract and standard show IC\(_{50}\) of 44 & 23 µg/ml correspondingly. The outcomes are revealed in Figure 4 and Table 3.

Figure 2: Scavenging activity of DPPH by \(H.\) hispidissimus

Anti-inflammatory activity

The extract tested at two different doses (100mg/kg & 250 mg/kg), exerted a considerable inhibitory effect on rat paw swelling after carrageenan administration with more than 50% inhibition for two doses. The maximum inhibition (58.97 & 71.73) was elicited by ethanolic extract of \(H.\) hispidissimus for (100 & 250mg/kg). Indomethacin used as reference

Figure 3: Hydroxy radical scavenging activity of \(H.\) hispidissimus

Figure 4: Superoxide radical scavenging activity
Table 2: DPPH activity of *H. hispidissimus*

| Concentration mcg/ml | Trial 1 | Trial 2 | Trial 3 | % Mean inhibition |
|----------------------|---------|---------|---------|-------------------|
| 5                    | 3.375   | 2.966   | 3.266   | 3.202             |
| 10                   | 5.316   | 5.564   | 5.345   | 5.409             |
| 15                   | 8.676   | 8.887   | 8.876   | 8.813             |
| 20                   | 13.485  | 13.786  | 13.696  | 13.656            |
| 25                   | 23.263  | 24.556  | 24.354  | 24.058            |
| 30                   | 28.761  | 28.556  | 28.842  | 28.720            |
| 35                   | 35.701  | 35.333  | 35.757  | 35.597            |
| 40                   | 49.572  | 50.465  | 50.486  | 50.175            |
| 45                   | 65.108  | 65.556  | 65.666  | 65.410            |
| 50                   | 78.875  | 80.497  | 80.299  | 79.891            |

Table 3: Half maximal inhibitory concentration of *H. hispidissimus*

| S no | Sample          | DPPH assay | Hydroxyl assay | Superoxide assay |
|------|----------------|------------|----------------|-----------------|
| 1    | H. hispidissimus | 42         | 2.8            | 38              |
| 2    | Ascorbic acid   | 12         | 1              | 24              |

Table 4: Percentage Inhibition of *H. hispidissimus*

| Sample                | % Inhibition of paw in time intervals (hr) |
|-----------------------|------------------------------------------|
|                       | 1          | 2          | 3          | 4          | 5          | 6          |
| Standard (Indomethacin)-20mg/kg | 25.31      | 42.90      | 57.5       | 61.95      | 71.74      | 72.35      |
| Test Ext -100 mg/kg    | 20.64      | 37.50      | 39.04      | 40.56      | 49.57      | 58.97      |
| Test Ext -250 mg/kg    | 25.30      | 40.58      | 55.27      | 55.64      | 62.04      | 71.73      |

Table 5: Anti-bacterial activity of *H. hispidissimus*

| Organism name   | Standard (Ciprofloxacian) | T1          | T2          | T3          | T4          |
|-----------------|---------------------------|-------------|-------------|-------------|-------------|
| S. aureus       | 24±0.11                   | 11±0.12     | 12±0.15     | 13±0.12     | 14±0.15     |
| B. subtilis     | 25±0.23                   | 11±0.13     | 12±0.15     | 13±0.22     | 14±0.13     |
| P. vulgaris     | 24±0.12                   | 11±0.12     | 12±0.16     | 12±0.23     | 12±0.14     |
| E. coli         | 25±0.22                   | 11±0.11     | 12±0.14     | 12±0.16     | 14±0.16     |

Figure 5: Anti-inflammatory activity of *H. hispidissimus*

The drug shows a similar inhibitory effect, it was able to exterminate paw oedema by 72.35%. The outcomes were revealed in Table 4 and Figure 5.

Anti-microbial activity

The *H. hispidissimus* extract was evaluated for anti-bacterial activity against, *Bacillus subtilis, Staphylococcus sp, Escherichia coli, Proteus Vulgaris*. The degree of inhibition as determined by values of the diameter of the inhibition zone (IZ) of respective extracts varied with the highest inhibition being recorded. *E. coli, B. subtilis* and *S. aureus* was the most susceptible bacteria to all plant extract; on the other hand, *P. aureginosa* and *C. Albicans* were the most resistant microorganism.

Table 5 shows *S. aureus* and *E. coli* have better mini-
Conclusions

The present study has been taken to evaluate the phytochemical and pharmacological activity of *H. hispidissimus*. The phytochemical studies revealed the presence of phenolic compounds, steroids, triterpenes, saponins and flavonoids. The data indicated that extracts from leaves of *H. hispidissimus* had antioxidant, anti-inflammatory and antimicrobial properties. The results revealed by antioxidant parameters shows that *H. hispidissimus* have antioxidant activity against various free radicals. Preventing the lysosomal membrane is one of the contributions to anti-inflammatory activity. Data of anti-inflammatory activity shows that plant extract exerted a considerable inhibitory effect on rat paw swelling after carrageenan administration and thereby inducing anti-inflammatory effect. The results indicate that promising activities of *H. hispidissimus*, and further, it could be subjected to drug progress and treatment of various infectious diseases.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

Funding Support

The authors declare that they have no funding support for this study.

References

Bhatt, K. R., Mehta, R. K., Shrivastava, P. N. 1977. A simple method for recording antiinflammatory effects on rat paw oedema. *Indian Journal of Physiology and Pharmacology*, 21(4):399.

Brand-Williams, W., Cuvelier, M. E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1):25–30.

Dabur, R., Gupta, A., Mandal, T. K., Singh, D. D., Bajpai, V., Gurav, A. M., Lavekar, G. S. 2008. Antimicrobial Activity Of Some Indian Medicinal Plants. *African Journal of Traditional, Complementary and Alternative Medicines*, 4(3):313–318.

Das, E. 2016. Introduction and importance of medicinal plants and herbs. Medicinal plants.

Evans, W. C. 2002. *Trease and Evans Pharmacognosy. Morphological and microscopical examination of drugs*, pages 3–603.

Fijesh, P. V., Ra, V. K. J., Vineesh, V. R., Padikkala, J. 2010. Antioxidant Activities of Hibiscus furcatus Roxb. ex DC. Extracts. *Research Journal of Biological Sciences*, 5(3):269–274.

Hafeez, A., Jain, U., Sajwan, P., Srivastava, S., Thakur, A. 2013. Evaluation of Carrageenan induced anti-inflammatory activity of ethanolic extract of bark of Ficus virens Linn. in swiss albino mice. *The Journal of Phytopharmacology*, 2(3):39–43.

Harborne, J. B. 1994. Indian Medicinal Plants. A Compendium of 500 Species. *Journal of Pharmacy and Pharmacology*, 46(11):935.

Hibiscus hispidissimus 1854. Database of plants of the Indian subcontinent -eflora of India. 4:521.

India biodiversity portal 2005. Hibiscus hispidissimus Griff.

Jeffrey, B. H. 1973. Phytochemical Methods. In and others, editor, *A Guide to Modern Techniques of Plant Analysis*, page 278. Springer Netherlands.

Khan, U. A., Rahman, H., Niaz, Z., Qasim, M., Khan, J., Tayyaba, Rehman, B. 2013. Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. *European Journal of Microbiology and Immunology*, 3(4):272–274.

Khan, Z. A., Naqvi, S. A., Mukhtar, A., Yar, N., Hussain, Z., Shahzad, S. A., Mansha, A., Ahmad, M., Zahoor, A. F., Bukhari, I. H., Ashraf-Janjua, M. R., Mahmood, S. 2014. Antioxidant and anti-bacterial activities of Hibiscus Rosa-Sinensis Linn flower extracts. *Pakistan Journal of Pharmaceutical Sciences*, 27(3):469–474.

Sofawara, A. 1996. Research on medicinal plants and traditional medicine in Africa. *The Journal of Alternative and Complementary Medicine*, 2(3):365–372.