Research Article **ISSN: 2349 – 7114**

Asian Journal of Research in Pharmaceutical Sciences and Biotechnology

Journal home page: www.ajrpsb.com

https://doi.org/10.36673/AJRPSB.2022.v10.i02.A05

EVALUATION OF ANTIOXIDANT ACTIVITY OF WATER, ACETONE FRACTION OF *HISBISCUS VITIFILIUS* FLOWERS

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ABSTRACT

Hisbiscus vitifilius L., a plant of the Convolvulaceae family, popularly known as morning glory, possesses numerous medicinal values. The present study aimed to explore the antioxidant activity and bioactive compounds of *Hisbiscus vitifilius* flowers. Antioxidant activity was determined using 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) ABTS (2, 2'-azinobis (3ethylbenzthiazoline-6-sulphonic acid), ferric reducing antioxidant power (FRAP), nitric oxide scavenging assay (NO), reducing power, hydroxy radical scavenging assay, superoxide radical scavenging (SOD), hydrogen peroxide radical assay, metal chelating activity as well as phosphomolypdenum and standard ascorbic acid (AA) assay. Based on the findings of this investigation, we can conclude that *Hisbiscus vitifilius* extract possesses various bioactive compounds and moderate antioxidant potentials, which may be a path to the discovery of traditional medicines and remedies for many critical diseases.

KEYWORDS

Antioxidant activity, DPPH, *Hisbiscus vitifilius* and Phytochemical screening.

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INTRODUCTION

Living organisms produce reactive oxygen species (ROS) as the result of cell metabolism. In low to moderate concentrations, ROS work in the physiological cell process; however, in high concentrations, ROS negatively modify the cell's components, such as lipids, DNA, and proteins. Antioxidants, which are chemical substances that can interact and neutralize free radicals, can prevent the harmful effects of ROS. Normally, every aerobic organism has a system that consists of

enzymatic and non-enzymatic antioxidants. However, in a pathological condition, the antioxidant system can be overwhelmed, which causes an imbalance between oxidants and antioxidants. In those cases, the human body needs an exogenous antioxidant to help ward off free radicals. Some exogenous synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) might have carcinogenic effect. Therefore, current research of exogenous natural antioxidants focuses on herbs. Spices and herbs have been identified as sources of diverse phytochemicals, and many of them have potent antioxidant activity[1-5]. The aim of this research is to use phytochemical analysis to evaluate antioxidant activity of chloroform and ethyl acetate extracts of the *Hisbiscus vitifilius* L flower.

MATERIAL AND METHODS

Plant material- Identification and authentication

Hisbiscus vitifilius flower was selectively removed from the plant in and around areas of Pudussery, Palakkad, Kerala and identified by a plant taxonomist. BSI/SRC/5/23/2022/Tech/631.

Preparation of *Hisbiscus vitifilius* flower extract

Hisbiscus vitifilius flower was washed, dried in a hot air oven at 40°C and subsequently ground into powder in an electric grinder. Delipidation was performed with Water and acetone soxhalation was performed with 95% Water and acetone was evaporated in a rotary evaporator at 40-50°C under reduced pressure. The yield of the flower extract was around 13.5% of dry weight.

Free Radical Scavenging Assays

The *in vitro* anti radical scavenging potential *Hisbiscus vitifilius* flower extract (100-500µg/ml) was determined using DPPH[6], ABTS[7], FRAP[8], Nitric oxide[9], Reducing power[10], hydroxy radical[11] superoxide scavenging[12], hydrogen peroxide[13], metal chelating activity as well as phosphomolypdenum assay[14,15].

Statistical analysis

All the assays were carried out in triplicate. Experimental results are expressed as mean ± standard deviation. The results were analyzed using one-way analysis of variance and the group means were compared using Duncan's multiple range test using SPSS version 16.

RESULTS AND DISCUSSION

Figure No.1 and Figure No.2 shows the effect *Hisbiscus vitifilius* flower extract Water and acetone on the DPPH and ABTS radicals present in the reaction mixtures. The extract at a concentration of 100 -500µg/ml, significantly scavenged of DPPH radicals with an IC_{50} value of 8.6,6.6µg/ml and ABTS radicals having IC_{50} values of 14.6, 12.9µg/ml.

Figure No.3 and Figure No.4 shows the effect of the FRAP power of the *Hisbiscus vitifilius* flower extract ethanol and chloroform with the increasing concentration was 14.6,12.4µg/ml. The scavenging of nitric oxide by *Hisbiscus vitifilius* was increased concentration of 19.1, 16.4µg/ml of *Hisbiscus vitifilius* 50% of nitric oxide generated by incubation was scavenged.

Figure No.5 shows the effect the reducing power *Hisbiscus vitifilius* flower extract ethanol and chloroform was increased in quantity of sample. The IC_{50} value of *Hisbiscus vitifilius* was 25.7, 22.9µg/ml respectively.

The results for hydroxyl scavenging assay are shown in Figure No.6. The concentrations for inhibition were found to be 28.4, 25.4 µg/ml for the *Hisbiscus vitifilius* respectively.

Figure No.7 and Figure No.8 shows the effect of the superoxide scavenging activity of *Hisbiscus vitifilius* flower extract ethanol and chloroform showed superoxide scavenging activity (IC_{50}= 40.7,38.4µg/ml), *Hisbiscus vitifilius* showed concentration dependent activity and the H_2O_2 scavenging effect at a concentration was 21.9, 20.5µg/ml.

Figure No.9 and Figure No.10 shows the effect of the metal chelating activity and phosphomolybdenum reduction of *Hisbiscus vitifilius* flower extract ethanol and chloroform to the quantity of the sample. The IC_{50} value of

Hisbiscus vitifilius was 46.2, 45.2µg/ml and 72.4,70.5µg/ml.

Discussion

The antioxidant property of plant confers their free radical scavenging potential their bioactive components and to understand the mechanism of action of their phytoconstituents[16]. In the present study, *Hisbiscus vitifilius* flower extracts scavenge DPPH and ABTS radicals in a concentration dependent manner. The amount of DPPH which is reduced may be estimated by observing a decrease in absorbance at 517nm. ABTS assay involves reduction of the color intensity of ethanolic solution containing pre-formed radical monocation of ABTS which is generated by oxidation of ABTS with potassium persulfate due to the radical scavenging activity of anti-oxidants present in the plants[17]. The change in intensity of the color is directly proportional to the antioxidant efficiency of the *Hisbiscus vitifilius* flower extract at a concentration of 100- 500µg/ml, the extract significantly scavenged of DPPH radicals (IC_{50}= 8.6,6.6µg/ml) , ABTS radicals (IC_{50}=14.6, 12.9µg/ml).

Some previous studies have also reported that the reducing power may serve as a significant indicator of potential antioxidant activity[18]. In this study, we used a FRAP assay because it is quick and simple to perform, and the reaction is reproducible and linearly related to the molar concentration of the antioxidant and FRAP assay was used by several authors for the assessment of antioxidant activity of various food product samples[19,20]. The reducing power of the *Hisbiscus vitifilius* increases with the increasing concentration 14.6,12.4µg/ml.

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological process including neurotransmission, vascular homeostasis, antimicrobial and antitumor activities. However, excess production of NO is associated with several diseases[21]. *Hisbiscus vitifilius* inhibited nitrite formation in a concentration dependent manner (100-500µg/ml). This may be due to the presence of antioxidant principles in the *Hisbiscus vitifilius* which complete with oxygen to react with nitric oxide. The scavenging of nitric oxide 19.1, 16.4µg/ml of *Hisbiscus vitifilius* of nitric oxide generated by incubation was scavenged.

The reducing power of the *Hisbiscus vitifilius* was evaluated by the transformation of Fe^{3+} to Fe^{2+} through electron transfer ability, which serves as a significant indicator of its antioxidant activity. Reductions are also reported to react with certain precursors of peroxide, thus preventing peroxide formation[22]. The presence of antioxidant substances in the compound samples causes the reduction of the Fe^{3+} ferric cyanide complex to the ferrous form. Therefore, Fe^{2+}can be monitored by measuring the formation of Perl's Prussian blue at 700nm. The IC_{50} value of *Hisbiscus vitifilius* was 25.7, 22.9µg/ml.

Hydroxyl radical scavenging capacity of *Hisbiscus vitifilius* is directly related to its antioxidant activity[23]. This method involves in vitro generation of hydroxyl radicals using Fe^{3+} /ascorbate/EDTA/ H_2O_2 system using Fenton reaction. The concentrations for inhibition were found to be 28.4, 25.4µg/ml for the *Hisbiscus vitifilius* respectively.

Superoxide radicals generated *in vitro* by the system was determined by the NBT photo reduction method. The decrease of absorbance at 560nm with the plant extract indicates the consumption of superoxide anion in the reaction mixture[24]. *Hisbiscus vitifilius* flower extract exhibited a maximum of superoxide scavenging activity (IC_{50}= 40.7, 38.4µg/ml).

Hydrogen peroxide is a weak oxidizing agent that inhibits the oxidation of essential thiol (-SH) groups directed by few enzymes. It can probably react with Fe^{2+} and possible Cu^{2+} ions to form hydroxyl radicals[25]. From the results, *Hisbiscus vitifilius* showed concentration dependent activity and the H_2O_2 scavenging effect at a concentration was 21.9, 20.5µg/ml.

Iron is an essential mineral for normal physiology, but an excess of it, may result in cellular injury[26,27]. The chelating ability of ferrous ions by the *Hisbiscus vitifilius* was estimated by the method. Ferrozine can quantitively form complexes with Fe^{2+}. In the presence of chelating agents, the

complex formation is disrupted with the result that the red color of the complex is decreased. The metal chelating activity of *Hisbiscus vitifilius* is present 46.2, 45.2µg/ml.

The phosphomolybdenum method is based on the reduction of M_0 (VI) to M_0 (V) by the antioxidant compounds and the formation of green phosphate/M_0 (V) complex with the maximal absorption at 695nm. The IC_{50} value of *Hisbiscus vitifilius* was 72.4,70.5µg/ml.

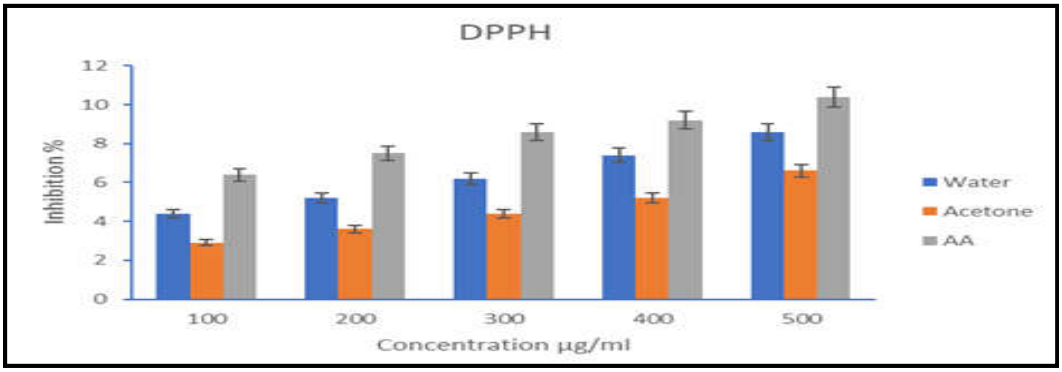

Figure No.1: Shows the DPPH effect of ethyl water and acetone flower extract of *Hisbiscus vitifilius*

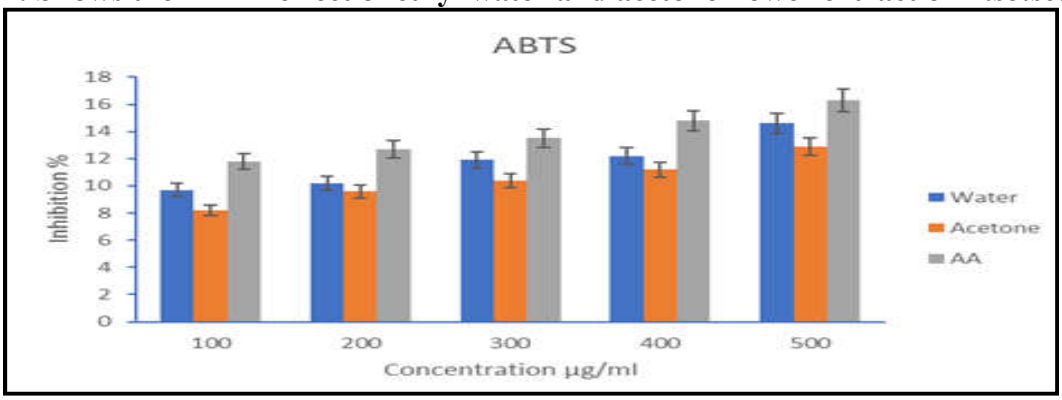

Figure No.2: Shows the ABTS effect of water and acetone flower extract of *Hisbiscus vitifilius*

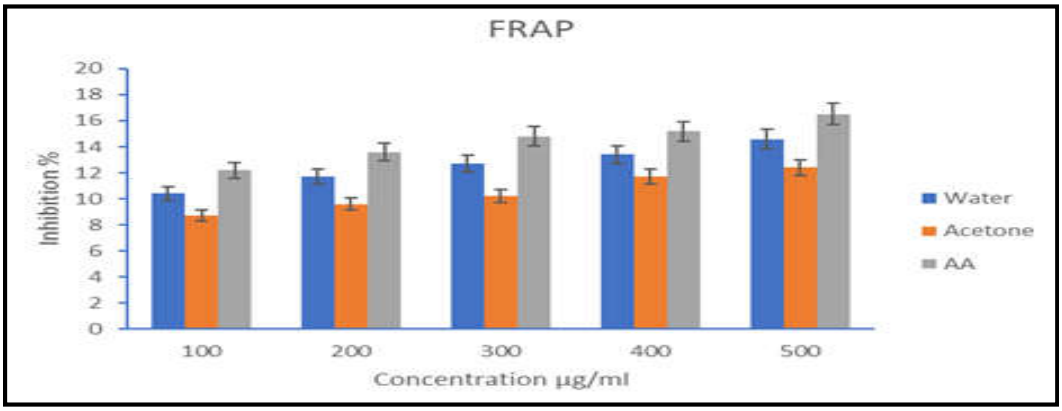

Figure No.3: Shows the FRAP effect of ethyl water and acetone flower extract of *Hisbiscus vitifilius*

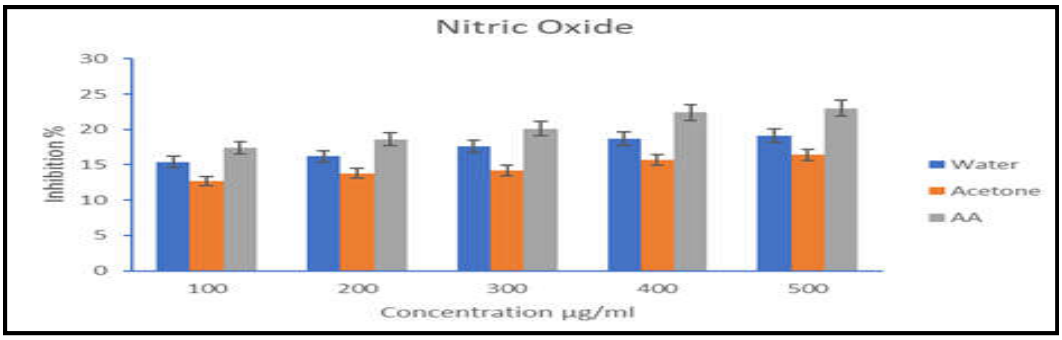

Figure No.4: Shows the Nitric oxide effect of ethyl water and acetone flower extract of *Hisbiscus vitifilius*

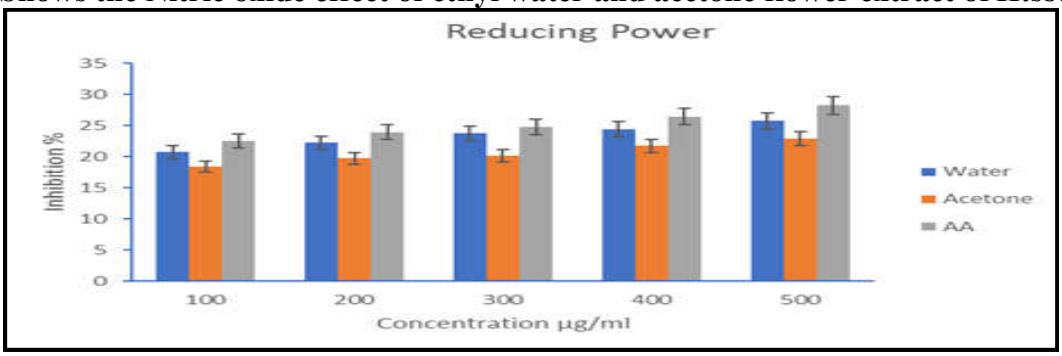

Figure No.5: Shows the Reducing power effect of ethyl water and acetone flower extract of *Hisbiscus vitifilius*

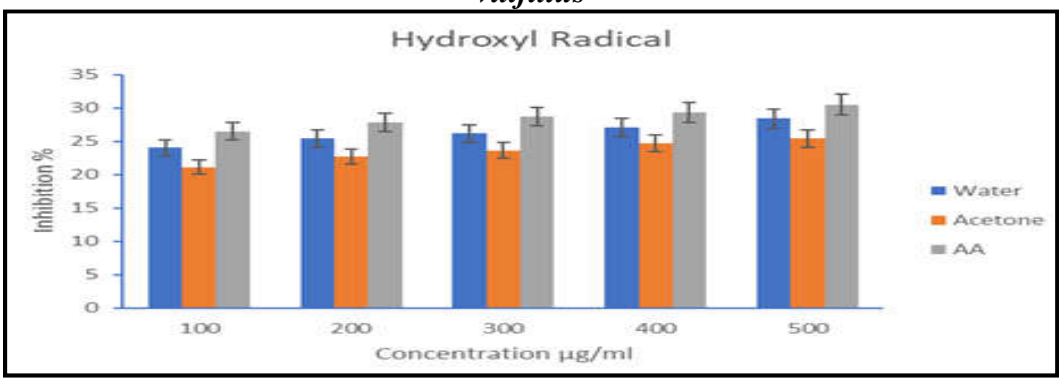

Figure No.6: Shows the Hydroxyl radical effect of ethyl Water and acetone flower extract of *Hisbiscus vitifilius*

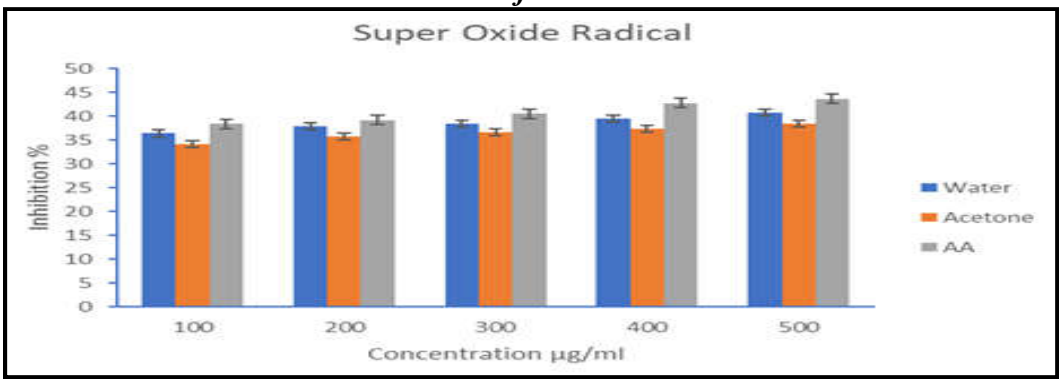

Figure No.7: Shows the superoxide radical effect of ethyl Water and acetone flower extract of *Hisbiscus vitifilius*

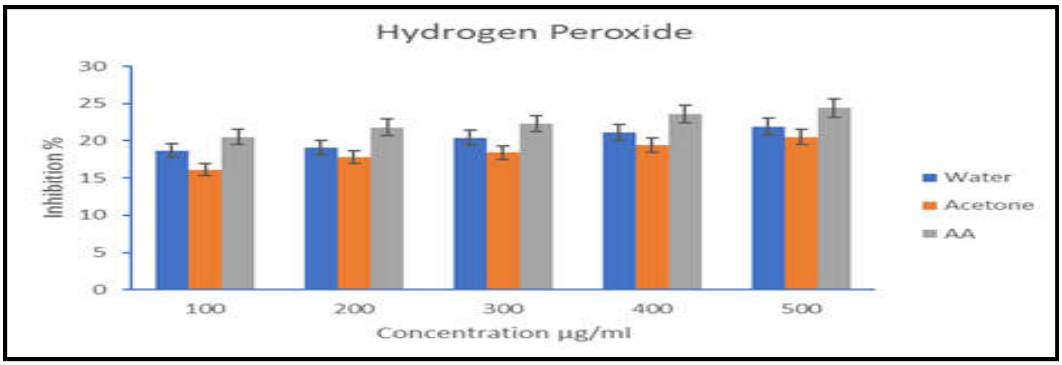

Figure No.8: Shows the hydrogen peroide effect of ethyl Water and acetone flower extract of *Hisbiscus vitifilius*

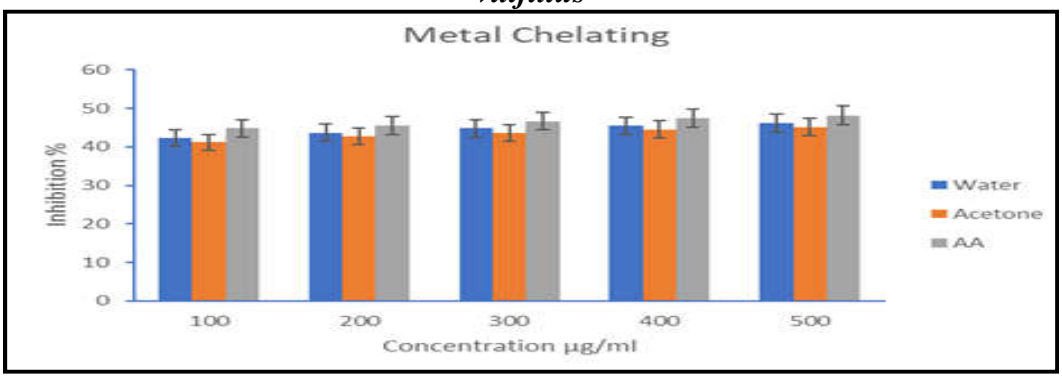

Figure No.9: Shows the Metal chelating effect of ethyl Water and acetone flower extract of *Hisbiscus vitifilius*

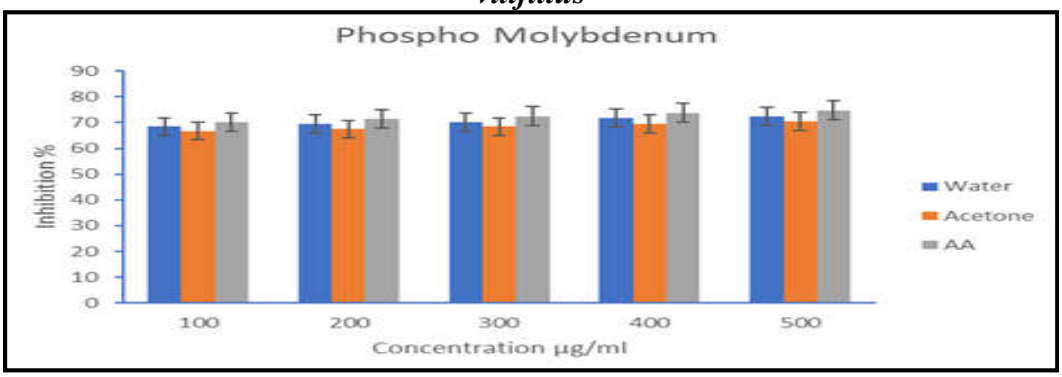

Figure No.10: Shows the Phospho molybdenum effect of Water and acetone flower extract of *Hisbiscus vitifilius*

CONCLUSION

Hisbiscus vitifilius species contain flavonoids and phenolic compounds which act as the primary anti-oxidants or free- radical scavengers. The presence of these compounds could be attributed to the potent anti-oxidant activity useful for the formulation of analgesic and anti-arthritic preparations.

ACKNOWLEDGMENT

We thank Dr. D. Kalpana. Principal, Sree Narayana Guru College, for the facilities provided is gratefully acknowledged.

CONFLICT OF INTEREST

There is no conflict of interest among all authors in this study.

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Please cite this article in press as: Baskaran K *et al*. Evaluation of antioxidant activity of water, acetone fraction of *Hisbiscus vitifilius* flowers, *Asian Journal of Research in Pharmaceutical Sciences and Biotechnology*, 10(2), 2022, 40-47.