International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 2(L); February 2018; Page No. 10427-10430
DOI: http://dx.doi.org/10.24327/ijcar.2018.10430.1766

Research Article

IN-VITRO ANTIOXIDANT ACTIVITY OF HIBISCUS PLANTIFOLIUS STEMS

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A R T I C L E I N F O

Article History:
Received 7th November, 2017
Received in revised form 10th December, 2017
Accepted 25th January, 2018
Published online 28th February, 2018

Key words:
Antioxidant; hepatoprotective; DPPH; nitric oxide

A B S T R A C T

Hibiscus plantifolius is a shrub with various medicianl uses and belongs to the Malvaceae family. Objective of the present work was to evaluate Methanol extract of Hibiscus plantifolius (MEHP) stems for possible in-vitro antioxidant activities. Antioxidant activity of the extract was evaluated by using Diphenyl picryl hydrazyl (DPPH) radical scavenging, Nitric oxide (NO) radical scavenging, super oxide free radical scavenging & Hydroxide free radical scavenging. Current investigation was reported that selected plants extracts such as methanolic extract of Hibiscus plantifolius was exhibited greater neutralization of DPPH*, NO*, SO* and OH* free radicals and also activity compared with standard curcumin. The antioxidant activity was exhibited due to presence of flavonoids and tannins, phenolic compounds which was present methanolic extract of Hibiscus plantifolius.

INTRODUCTION

Oxidative stress resulting from the toxic effects of free radicals on the tissue plays an important role in the pathogenesis of various pathological conditions such as ageing process, anemia, arthritis, asthma, inflammation, ischemia, mongolism, neurodegeneration, Parkinson's disease, and perhaps dementia. Antioxidants are molecules that inhibits the oxidation of other molecurs and are radical scavengers, which protect the human body against free radicals[1] . Free radical also induces liver damage. Likewise, metabolism of certain drugs like paracetamol, produce free radicals, which cause liver damage. Antioxidants may offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by other mechanisms and thereby help in preventing the free radical induced diseases[2].

The largest exocrine gland of our body, the liver, plays vital functions in association with homeostasis of the body. Anabolic and catabolic pathways of nutrients that we consume and de-toxification of ingested food based chemicals are taken care by our liver[3]. The variety of ingested chemicals induces liver injury by mostly causing oxidative stress in hepatic tissue and accounts for numerous diseases, including cancer. Considering the fact of widespread and casual abuse of liver, like environmental toxins, prescription and over-the-counter drug use, which lead to hepatitis, cirrhosis and liver disease, more research light is thrown in the use of antioxidants for prevention and/or amelioration of hepatic injury. This amelioration process is often referred to as "The chemoprevention", and a large body of evidence from various experiments has supported its efficiency[4].

Hibiscus plantifolius (Maple-Leaved Mallow), is a species of flowering tree in the mallow family, Malvaceae, that is native to the India and Sri Lanka. In Sri Lankan texts, the plant is widely known by its synonym H. eriocarpus. The tree is about 8m tall. Leaves are cordate at base; hairy; trilobed. Flowers show axillary panicles where flowers show typical Hibiscus flower colors, pink with dark center. Fruit is a capsule. Common Names for this plant in kannada:- Bili daasavaala, Daasaala, Daasaani & in telugu :- Telugu - Kondabenda, Kondagogu, Kondajana punara.

MATERIALS AND METHODS

Plant material

2 kg of the stem of Hibiscus plantifolius[5] were collected from the Thirumala forest in Andhra Pradesh State, India, in the months of June and July 2017. The stem of Hibiscus plantifolius was washed and allowed to dry for 15 days. The dried stem was then ground to fine powder by using the laboratory Hammer mill. Powdered samples were stored desiccators until required for extraction.

Preparation of Hibiscus plantifolius extract

The powdered materials of Hibiscus plantifolius was extracted individually with petroleum ether, chloroform and methanol using soxhlet apparatus[6], each for 18 hours. The extracts were

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concentrated using rotary evaporator till free from the solvents and obtained yield was respectively 1g/kg, 12 g/kg and 25 g/kg respectively. The remain crude material was macerated with distilled water and obtained yield was 53 g/kg.

Phytochemical analysis

Phytochemicals[7] are naturally present in the plants and shows biologically significance by playing an essential role in the plants to defend themselves against various pathogenic microbes by showing the antimicrobial activity by inhibition or killing mechanisms. The secretion of these compounds is varying from plant to plant some produce more and some produce in minimal quantity. Sometimes they can be harmful and sometimes they can be very helpful. There is evidence from laboratory studies that phytochemicals in fruits and vegetables may reduce the risk of cancer, possibly due to dietary fibers, polyphenol antioxidants and anti-inflammatory effects. Specific phytochemicals, such as fermentable dietary fibers, are allowed limited health claims by the US Food and Drug Administration.

Hence, preliminary chemical tests were carried out for extracts and isolated fractions to identify different Phytoconstituents such as carbohydrates, amino acids, proteins, fixed oils and fats, triterpenoids, alkaloids, tannins, flavonoids, glycosides, saponins, tannins and phenolic compounds (Harborne. JB,1973)

RESULTS

S. No	Group of Phytoconstituents	Extracts			
		Petroleum ether	Chloroform	Ethanol	Aqueous
1	Carbohydrates	-	+	+	+
2	Amino acids		-	-	-
3	Proteins	+	-	-	-
4	Fats and oils	+	-	-	-
5	Alkaloids	-	-	-	-
6	Terpenoids	-			-
7	Flavonoids	-		+	-
8	Cardiac glycosides	-	+	-	
9	Saponin	-	+	-	+
10	Tannins and Phenolic compounds	-	-	+	-

Evaluation of antioxidant activity

DPPH* free radical scavenging activity

Each concentration of one ml of was mixed with 3 ml of a DPPH-methanol solution (40µg/ml) .This was kept for20 minutes for the reaction to occur. Then the absorbance was determined at 517 nm and calculates the percentage of inhibitions using the following equation:

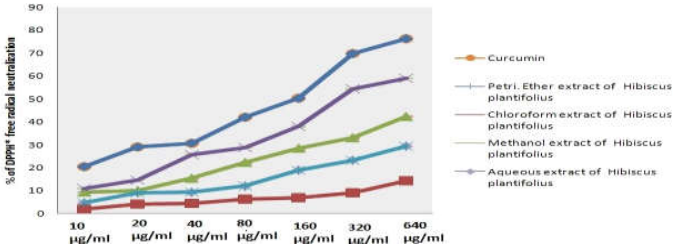
% inhibition = [1- (Ab. of Ssample / Ab. of control)] x 100.

In DPPH scavenging activity[8], all the extracts showed concentration-dependent activity. The results are tabulated below:-

Result are mean ± SD for 6 animals; Significant at *** P< 0.001 **P<0.05 compare to control.

S.No	Con. (µg/ml)	10	20	40	80	160	320	640
	Extracts	% of DPPH* free radical neutralization						
1	Curcumin	20.5±1.2	29.11±1.1	30.8±2.2	42.07±2.4	50.3±2.5	69.8±2.1	***76.3±2.3
2	Ether	2±0.3	4.1±1.2	4.6±1.3	6.3±1.1	7.2±2.2	9.1±2.1	**14.3±0.87
3	Chloroform	9.2±0.76	9.9±0.89	15.3±1.1	22.2±1.2	28.4±1.3	33.3±2.1	**42.3±1.5
4	Methanol	10.9±1.3	14.5±1.2	25.6±2.3	28.7±2.4	37.8±2.6	54.11±3.2	**58.8±1.8
5	Aqueous	4.8±0.34	9.1±0.87	9.3±0.98	12±1.2	18.9±1.4	23.2±2.2	29.3±1.3

Effect of Hibiscus plantifolius stem extracts on DPPH* free radicals



NO* free radical scavenging activity

One ml of each concentration of test sample was taken and to this add 1ml of Sodium nitroprusside solution. This solution was incubated at 37°c for 3 hours. Add 0.3ml of Griess reagent to above 1 ml of aliquot of incubation solution. The absorbance was measured at 570 nm using UV spectrophotometry[9].

The percentage inhibition of radical by test sample was calculated using the formula:

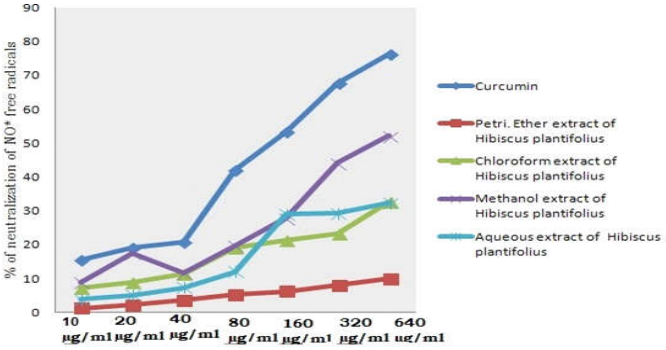
% inhibition = [1- (Ab. of Sample / Ab. of control)] x 100.

S.No	Con. (µg/ml)	10	20	40	80	160	320	640
	Extracts	% of NO* free radicals neutralization						
1	Curcumin	15.5±2.2	19.11±0.1	20.8±1.2	42.07±2.4	53.3±1.5	67.8±1.1	***76.3±1.3
2	Ether	1.2±0.2	2.1±0.2	3.6±0.3	5.3±0.1	6.2±1.2	8.1±1.1	9.9±0.9
3	Chloroform	7.2±0.7	8.9±0.8	11.3±0.9	19.2±0.2	21.3±0.3	23.3±0.9	*32.6±0.5
4	Methanol	9.0±0.3	17.5±0.2	11.6±0.3	19.7±0.4	27.8±1.6	44.1±0.2	**52.1±0.8
5	Aqueous	3.8±0.3	5.1±0.8	7.3±0.9	12±0.2	28.9±0.4	29.2±0.2	32.3±0.3

Results: Effect of Hibiscus plantifolius stem extracts on NO* free radicals.

Results are mean ± SD for 6 animals; Significant at *** P< 0.001 **P<0.01, *P<0.05 compare to control

Effect of Hibiscus plantifolius stem extracts on NO* free radicals



Superoxide Anion (SO*) free radical scavenging activity

Super oxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitro blue tetrazolium (NBT).

In this experiments, the superoxide radicals were generated in 3 ml of Tris-HCI buffer (16 mM, pH 8.0) containing I ml of NBT (50 mM) solution, 1 ml NADH (78 mM) solution and sample solution of different concentration of MEHP in water. These all contents were mixed. The reaction was started by adding 1 ml of phenazine methosulphate (PMS) solution (10 mM) to the mixture.

The reaction mixture was incubated at 25°C for 5 minutes, and the absorbance at 560 nm in a spectrophotometer was measured against blank samples. Curcumin was used as a control[10].

Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

% inhibition = [1- (Ab. of Sample / Ab. of control)] x 100.

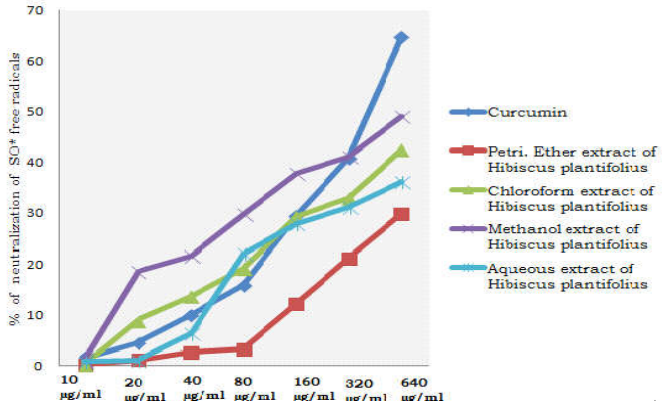
Results

Effect of Hibiscus plantifolius stem extracts on SO* free radicals

S.No	Con. (µg/ml)	10	20	40	80	160	320	640
	Extracts	% of SO* free radicals neutralization						
1	Curcumin	1.5 ±0.2	4.5± 0.3	10±0.2	15.8±0.95	29.5±0.45	40.9±0.67	***64.7±0.93
2	Ether	0.2±0.01	1.1±0.1	2.6±0.2	3.3±0.2	12.2±1.2	21.1±0.1	29.9±0.8
3	Chloroform	0.2±0.01	8.9±0.6	13.7±0.7	19.2±0.1	29.3±0.1	33.1±0.8	**42.6±0.3
4	Methanol	1.1±0.02	18.5±0.1	21.6±0.4	29.7±0.3	37.8±1.6	41.1±0.2	**49.1±0.2
5	Aqueous	0.8±0.2	1.1±0.7	6.3±0.9	22±0.5	28 ±0.2	31.1±0.2	36.3±0.2

Results are mean ± SD for 6 animals; Significant at *** P< 0.001 **P<0.01 compare to control

Effect of Hibiscus plantifolius stem extracts on SO* free radicals



Hydrogen Peroxide (OH*) Free Radical Scavenging Activity

Different concentrations of extract were dissolved in 3.4 ml of 0.lM phosphate buffer (having p^H 7.4) and were mixed with 600 µL of 43 mM solution of hydrogen peroxide (30%).

The absorbence value at 230 run of the reaction mixture was recorded at 10 min intervals between zero and 40 minutes for each concentration.

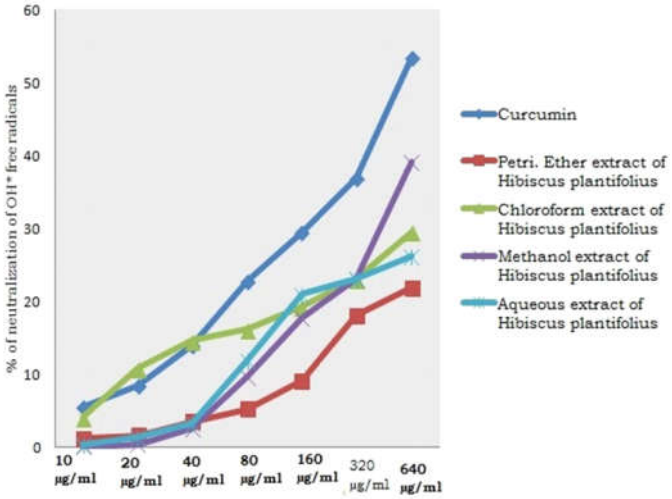
% inhibition = [1- (Ab. of Sample / Ab. of control)] x 100.

S.No	Con. (µg/ml)	10	20	40	80	160	320	640
	Extracts	% of OH* free radicals neutralization						
1	Curcumin	5.6±0.5	8.5±0.6	14±0.2	22.8±0.2	29.5±0.5	36.9±0.7	**53.4±09
2	Ether	1.2±0.1	1.7±0.1	3.6±0.2	5.3±0.1	9.2±1.2	18.1±0.1	21.9±0.8
3	Chloroform	4.2±0.01	10.9±0.5	14.7±0.4	16.2±0.2	19.3±0.1	23.1±0.3	*29.6±0.2
4	Methanol	0.1±0.02	0.5±0.01	2.6±0.4	9.7±0.3	17.8±1.6	23.1±0.2	*39.1±0.2
5	Aqueous	0.5±0.2	1.5±0.7	3.3±0.4	12±0.3	21 ±0.4	23.1±0.1	26.3±0.2

Effect of Hibiscus plantifolius stem extracts on OH* free radicals

Results are mean ± SD for 6 animals; Significant at *** P< 0.001 **P<0.01, *P<0.05 compare to control

Effect of Hibiscus plantifolius stem extracts on OH* free radicals



Calculation of 50% inhibition concentration

The graph was extrapolated between concentrations of the plant extracts and % of inhibition to find out the 50% inhibition concentration. The extracts were exhibited dose dependent neutralization of DPPH*, NO*, SO* and OH* free radicals and activity was compared with standard curcumin (as shown in the result tables).

The IC 50 of 310 µg, 620 µg, greater than 640 µg/ml methanolic extract of Hibiscus plantifolius stem against of DPPH*, NO*, SO* and OH* free radicals, respectively. This indicates the methanolic extract Hibiscus plantifolius stem exhibited antioxidant activity.

DISCUSSION

Many scientific studies are revealed that the antioxidative activity of herbal plants due to presence of phytochemicals such as flavonoids and saponins (Ardestani A and Yazdanparast R 2007) & in order to ascertain whether there is any link between the Methanomedicinal applications of Hibiscus plantifolius and its antioxidant activities, different methods were employed to evaluate the free radical scavenging and antioxidant activities of methanol extract agents for thousands of years and an impressive number of modern drugs have been developed / isolated natural resources, may based on their use in traditional of stem of Hibiscus plantifolius (MEHP). Current investigation was reported that selected plants extracts such as methanolic extract of Hibiscus plantifolius[11] was exhibited greater neutralization of DPPH*, NO*, SO* and OH* free radicals and also activity compared with standard curcumin.

CONCLUSION

Nature has been a source of medicinal medicine. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since the ancient times. The antioxidant activity was exhibited due to presence of flavonoids and tannins, phenolic compounds which was present methanolic extract of Hibiscus plantifolius. Present study shows that poly-phenols content in the methanolic stem extracts of Hibiscus plantifolius is high and these extracts exhibit strong antioxidant activities compared to that of the standard compounds. It is an easily available plant for natural remedies.

References

Ames BN, Shigenaga MK, Hagen TM, *Proc Natl Acad Sci* USA, 1993, 90, 7915-7922.

Dean RT, Davies MJ, *Trends Biochem Sci*, 1993, 18, 437-441.

Ceruti P, Lancet, 1994, 344, 862-863

Lee JK, Min DB, Comprehensive Review of Food Sci and Food Safety, 2004, 3, 21-33

A Textbook of Pharmacognosy and Phytochemistry. Kumar GS and Jayaveera KN. S. Chand & Company Pvt. Ltd. 2014.

Akindahunsi AA, Olaleye MT. Toxicological investigation of aqueous methanolic extract of Hibiscus sabdariffa L. *J Ethnopharmacol*. 2003; 89: 161-16

Rajan S, Gokila M, Jency P, Brindha P, Sujatha RK. *Int J Curr Pharm Res*, 2011, 3(2), 65-70

Ananth A, Rajan S, *European J Biomed and Pharmaceutical Sci*, 2015, 2(2), 281-294.

Jonathan Y, Aus *J Basic and Appl Sci,* 2009, 3(4), 3975-3979.

Ayoola GA, Coker HAB, Adesegun SA, Adepoju BAA, Obaweya K, Ezennia EC, Atangbayila TO, *Trop J Pharma Res,* 2008; 7 (3),1019-1024

Salem MZM, Perez JO, Salem AZM. Studies on biological activities and phytochemicals composition of Hibiscus species- A Review, *Life Science Journal* 2014;11(5): 1-8 27

How to cite this article:

Sowjanya K *et al* (2018) 'In-Vitro Antioxidant Activity of Hibiscus Plantifolius Stems', *International Journal of Current Advanced Research,* 07(2), pp. 10427-10430. DOI: http://dx.doi.org/10.24327/ijcar.2018.10430.1766

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