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PHYTOCHEMICALSCREENING ANDANTI-OXIDANT ACTIVITY OFAERIAL PARTS OF *HIBISCUS SURATTENSIS* LINN

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ABSTRACT

The present study was carriedout to find the phytochemical constituents and*in-vitro* antioxidant potentials of *Hibiscus surattensis* Linn. The dried powdered leaves of *Hibiscus surattensis*Linn were extracted using petroleum ether, chloroform, ethyl acetate and methanol using a Soxhlet extractor and preliminary phytochemical screening was carried out using standard protocols. All the extracts were evaluated for their potential antioxidant activities using test such asDPPH, hydroxyl radical and superoxide anion radical scavenging abilities. Preliminary screening showed the presence of bioactive compounds especially phenolics, tannins and steroids in all extracts. The results of the present study shows that,the aerial parts of *Hibiscus surattensis*Linndisplayed strong anti-oxidant activity and thus it is a good source of antioxidant constituents.

INTRODUCTION

Nature's fauna and flora gave us a complete store of remedies to treat the ailments of suffering humankind. In recent years herbal drugs are quickly becoming popular as an alternative therapy. As plants maintainhuge amounts of antioxidants to control the oxidative stress due to sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity. Plants contain a wide variety of free radical scavenging molecules, such as phenolic compounds[1], nitrogen compounds, vitamins, terpenoids (including carotenoids) and some other endogenous metabolites, which are rich in antioxidant activity[2]. Natural antioxidant have a wide range of biochemical activities including inhibition of reactive oxygen species (ROS) generation, direct or indirect scavenging of free radicals and alteration of intracellular redox potential[3]. Oxidative stress occurs when there is imbalance between two process, ie generation of reactive species with oxygen and endogenous antioxidant processes. Antioxidants fight against free radicals and protect us from various diseases. The use of antioxidants found naturally has gained much attention from consumers because they are considered safe to use than synthesized antioxidants. Recently there has been a worldwide trend towards the usage and intake of natural antioxidants present in different parts of certain plants due to their phytochemical constituents[4,5]. The intake of natural antioxidants has been associated with the reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with ageing[6,7]*Hibiscus L*. is a type of genus of

the tribe *Hibisceae* of the family *Malvaceae*. The genus contains about 300 species that grows in tropical and sub-tropical areas throughout the world. Most Hibiscus species have a remarkable colour pattern with the base of corolla forming a deepcolored heart. Some species are of value as source of foods and medicines and as ornamental plants.[8]

Hibiscus surattensis Linn *(H. surattensis)* is a medicinal plant belongs to Malvaceae family. *Hibiscus surattensis*Linn is an indigenous scrambling annual commonly known as Wild Sour. All parts, including the weak stems and leaf stalks are covered with small downwardpointing soft prickles and hairs. Pairs of oval leafy stipules beside the stalks are characteristic. Leaves are usually reddish and flowers with very showy orangeyellow, over 7 cm across, petals dark redmaroon at the centre. The 6 or more outer sepals divide into a narrow erect lobe and a broader oval lobe. The 5 inner sepals lengthen to 2.5 cm in fruit, tip pointed, covered with hairs. Various medicinal properties of Hibiscus have been observed. For example, in West Africa, *H. surattensis* extract is utilized with other species in malaria treatment, with the leaves ingested as a decoction. In Nigeria, *H. surattensis* flowers are used for the treatment of hypertension. The stems and leaves of *H. surattensis* are used as a lotion to treat venereal disease and ureteritis in India and the eastern regions of South Africa.[9] Anti-inflammatory, antifungal, antipyretic, and antihelminthic activities were identified from stems and root extracts of *H. taiwanensis*. Flowers of *H. tiliaceus* are widely used for birth control and for treating skin infections. *H. rosa-sinensis* extracts exhibit anticancer, antioxidant and antibacterial activities. Sorrel (*H. sabdariffa*), a medicinal herb commonly uses to make drink and pickle, is used as a folk medicine for the treatment of hypertension, liver diseases, and fever. *H. sabdariffa* has been reported to possess antihypertensive, antioxidant, anti-cancer, anticlastrogenic, hypolipidaemic, hepatoprotective, antistress, antispasmodic, diuretic and antidiarrheal activities. The decoction of the plant seeds is given to augment or induce lactation in poor let-down and maternal mortality. Antibacterial and antioxidant activities were found from other Hibiscus species namely *platanifolius*, *Cannabinus*, *mutabilis*, *esculentus* and *tiliaceus*.[10].Thus in the light of knowledge that *Hibiscus surattensis* Linn is having wide folklore uses, we intend to evaluate the antioxidant activities of the various extracts of *Hibiscus surattensis* Linn using *in vitro* models.

Materials and Methods:

Plant Material

The aerial parts of *Hibiscus surattensis*Linn were collected from Tirunelveli district, Tamilnadu, India during the month of March 2016. The plant was identified and authenticated by Mr. Chelladurai, Research Officer- Botany, Central Council for Research in Ayurveda and Siddha, Government of India (Ref No:- DCP/CH/AN02).

Chemicals and instruments

DPPH were purchased from Sigma – Aldrich, USA. 2-deoxy-2-ribose, gallic acid, curcumin, Ascorbic acid and quercetin were purchased from Himedia Labs., Pvt.Ltd, Mumbai, India. All other drugs and chemicals used for the work were purchased commercially and were of analytical grade. U-V spectrophotometer Shimadzu was used to measure the absorbance.

Extraction

The aerial parts of *Hibiscus surattensis*Linn were collected, shade dried, powdered mechanically and sieved through No. 20 mesh sieve. About 100g of the powdered aerial part is first extracted with petroleum ether (PEH, 40º-60ºC) and then consecutively with chloroform (CEH), ethyl acetate (EAEH) and methanol (MEH). The percentage yield of the extracts is listed in table 1.

Phytochemical screening of the extracts: Chemical tests were carried out for the all the extract of *Hibiscus surattensis*Linn for the presence of phytochemical constituents like phenols, tannins, saponins, flavonoids, terpenoids, alkaloids, glycosides and steroids[11,12]

Antioxidant activity

DPPH radical scavenging activity[13:] DPPH (1-diphenyl-2-picrylhydrazyl) assay gives an account on the free radical scavenging ability[13]. Briefly about 1ml (0.1mM) of DPPH solution prepared in methanol was added to 3 ml of test or standard (Gallic acid) solution at different concentration (0.25-64µg/ml). The mixture was incubated in dark at 30º C for 30 min and the absorbance measured at 517 nm and percentage inhibition calculated. A control reaction was carried out without the test sample.

Hydroxyl radical scavenging activity[14]

Hydroxyl radical scavenging activity of the extract is determined by its ability to scavenge the hydroxyl radicals produced by the EDTA-Fe^{3+}-H_2O_2-ascorbic acidsystem by a reaction known as Fenton reaction[14].The reaction mixture amounts to a final volume of 1.0 ml which contains 100 µl of 2-deoxy2-ribose (28 mM) in phosphate buffer solution (20 mM, pH 7.4), 500 µl of the extracts at various concentrations (10-160 µg/ml) in buffer solution, 200 µl of 1.04 mM EDTA and 200 µM $FeCl_3$ (1:1v/v), 100 µl of H_2O_2 (1.0 mM) and 100 µl of ascorbic acid (1.0 mM). Test samples were incubated at 37°C for 1 h. Thiobarbituric acid test was used to assess the free radical damage inflicted on the substrate, deoxyribose. The positive control which was used for this assay was quercetin (10-160 µg/ml).The percentage inhibition of the extracts and standard were calculated.

Superoxide radical scavenging activity[15]: The superoxide radicals are generated in a phenazinemethosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by the reduction of nitrobluetetrazolium (NBT)[15]. The superoxide radicals were generated in this experiment in 3 ml of Tris–HCl buffer (16 mM, pH 8.0) containing 78 mM NADH, 50 mM NBT, 10 mM PMS and extracts to be tested at different concentrations (10-160 µg/ml). The colour reaction between superoxide radicals and NBT was detected at 560 nm and the percentage inhibition calculated. Positive control used was Ascorbic acid (10-160 µg/ml).

Calculation of 50% inhibitory concentration (IC_{50}).The concentration (µg/ml) of the extract required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the extracts. Percentage inhibition *(I%)* was calculated using the formula:

$$I\% = \frac{Ac - At}{Ac} x100$$

where A_c is the absorbance of the control and A_t is the absorbance of the test sample.

2.14. Statistical analysis*:* All the experiments were carried out in triplicate and results expressed as mean ± SEM. Significant differences among means of samples were evaluated by one-way analysis of variance (ANOVA).

RESULTS

Phytochemical screening of the extract: Phytochemical analysis showed the presence of phenolics, terpenoids, tannins, flavanoids and steroids in the extract(Table.2).

DPPH radical scavenging activity: DPPH radical scavenging of various extracts of the leaves of *Hibiscus surattensis*Linn was investigated and results were shown(Table.3). All the extracts showed a dose dependent scavenging activity, of which the methanolic extract showed the highest activity. The gallic acid which was used as standard had higher scavenging activity than any of the extracts.The highest activity was shown by MEH (IC_{50} = 2.19±0.120) and the order of decreasing scavenging ability is MEH>EAEH (16.52±0.165) > CEH (31.74±0.065). All extracts showed significant ($P<0.05$) scavenging ability when compare with standard gallic acid (IC_{50} = 1.82±0.132).

Hydroxyl radical scavenging activity: The extracts and the standard (quercetin) inhibited the formation of hydroxyl radical in a dose dependent manner (Table.5) The MEH(IC_{50} = 48.87±0.104) showed the maximum quenching ability followed by EAEH (IC_{50} = 77.21±0.646) andCEH (IC_{50} = 136.56±0.578). The *in vitro* radical scavenging ability of the extracts were found to be significant ($p<0.05$) when compared with the standard quercetin(IC_{50} = 24.85±0.725).

Table 1: Percentage yield of various extracts

Extracts	% Yield (w/w)
PEH	4.8
CEH	1.5
EAEH	2.3
MEH	4.2

PEH:- Pet Ether Extract of *Hibiscus surattensis*; CEH:- Chloroform Extract of *Hibiscus surattensis*EAEH:-Ethyl Acetate Extract of *Hibiscus surattensis*; MEH:-Metanolic Exract of *Hibiscus surattensis*

Table 2: Phyto chemical screening of various extracts

Phytochemicals	PEH	CEH	EAEH	MEH
Tannins and phenolics	+	+	+	+
Saponins	+	-	-	+
Flavonoids	-	-	+	+
Terpenoids	-	+	+	+
Alkaloids	-	+	+	+
Glycosides	-	-	-	-
Steroids	+	+	+	+
Proteins	-	+	+	+

PEH:- Pet Ether Extract of *Hibiscus surattensis*; CEH:- Chloroform Extract of *Hibiscus surattensis*EAEH:-Ethyl Acetate Extract of *Hibiscus surattensis*; MEH:-Metanolic Exract of *Hibiscus surattensis*

Table 3 DPPH Radical scavenging activity of *Hibiscus surattensis*

CONC µg/ml	Percentage inhibition (%)				
	PEH	CEH	EAEH	MEH	Gallic acid
0.25	-	-	-	5.66±0.323	09.67±0.412
0.5	-	-	-	17.44±0.926	20.38±0.221
1	-	-	-	35.27±0.261	32.33±0.102
2	-	-	05.21±0.120	49.42±0.356	54.52±0.182
4	06.23±0.312	08.92±0.245	16.12±0.185	63.92±0.155	71.75±0.202
8	15.10±0.311	21.62±0.124	35.09±0.212	79.02±0.058	83.96±0.092
16	18.82±0.436	35.26±0.157	49.96±0.223	86.59±0.169	94.50±0.071
32	25.16±0.922	50.41±0.057	69.62±0.162	92.47±0.132	98.47±0.232
64	32.88±0.062	60.12±0.138	88.49±0.147	94.49±0.069	100
IC_{50} µg/ml	#	31.74±065	16.52±0.165	02.28.±0.132	01.83±0.116

PEH: Pet Ether Extract of *Hibiscus surattensis*; CEH:- Chloroform Extract of *Hibiscus surattensis* EAEH:-Ethyl Acetate Extract of *Hibiscus surattensis*; MEH:-Metanolic Exract of *Hibiscus surattensis*. All values determined were mean ± SEM; *n*= 3. *P < 0.05 when compared with standard

Table 4 Hydroxyl radical scavenging activity of *Hibiscus surattensis*

CONC µg/ml	PERCENTAGE INHIBITION (%)				
	PEH	CEH	EAEH	MEH	QUERCETIN
10	05.44± 0.181	08.97±0.117	10.47±0.029	13.55±0.265	20.62±0.106
20	11.76±0.048	17.04±0.759	21.15±0.162	26.24±0.195	47.21±0.202
40	18.40±0.210	35.64±0.262	40.94±0.093	47.23±0.187	58.65±0.089
80	20.51±0.225	44.38±0.241	50.67±0.292	60.34±0.210	72.13±0.138
160	24.35±0.129	52.44±0.187	60.50±0.106	78.43±0.274	88.43±0.309
IC_{50} µg/ml	#	136.56±0.578	77.21±0.646	48.87±0.104	24.85±0.725

PEH: Pet Ether Extract of *Hibiscus surattensis*; CEH:- Chloroform Extract of *Hibiscus surattensis* EAEH:-Ethyl Acetate Extract of *Hibiscus surattensis*; MEH:-Metanolic Exract of *Hibiscus surattensis*. All values determined were mean ± SEM; *n*= 3. *P < 0.05 when compared with standard

Table 5 Super oxide radical scavenging activity of *Hibiscus surattensis*

CONC µg/ml	PERCENTAGE INHIBITION (%)				
	PEH	CEH	EAEH	MEH	ASCORBIC ACID
10	05.81± 0.322	09.25±0.258	11.24±0.145	16.61±0.098	26.30±0.173
20	12.81±0.398	15.23±0.021	23.57±0.247	29.67±0.174	42.51±0.163
40	20.31±0.073	32.13±0.154	37.25±0.152	47.37±0.167	56.33±0.104
80	33.29±0.025	45.02±0.044	51.55±0.069	68.14±0.530	71.58±0.231
160	38.22±0.127	54.97±0.241	65.48±0.126	81.50±0.212	84.55±0.248
IC_{50} (µg/ml)	#	126.32±0.074	77.31±0.146	45.06±0.106	30.10±0.432

PEH:- Pet Ether Extract of *Hibiscus surattensis*; CEH:- Chloroform Extract of *Hibiscus surattensis* EAEH:-Ethyl Acetate Extract of *Hibiscus surattensis*; MEH: Metanolic Exract of *Hibiscus surattensis*. All values determined were mean ± SEM; *n*= 3. *P < 0.05 when compared with standard

Superoxide radical scavenging activity: The superoxide radical scavenging ability was found to increase with increase in concentration of the extract. The MEH (IC_{50} = 45.06±0.106) was found to be an efficient scavenger of superoxide anion radical generated from PMS-NADH system *in vitro* and the activity was significant (P<0.05) when compared to that of standard ascorbic acid (IC_{50} = 30.10±0.432). The scavenging effects of extracts on the superoxide anion radical decreased in order MEH>EAEH (IC_{50} = 77.31±0.146) >CEH (IC_{50} = 126.32±0.074) (Table.4)

4. DISCUSSION:

Phytochemical screening: Various bioactive components such as flavanoids, tannins, phenolics, terpenoids and steroids were prominently revealed during the preliminary phytochemical screening. Phenolics,tannins and steroids were present in all the extracts whereas glycosides were absent in all the extracts. Alkaloids, terpenoids and proteins were absent in petroleum ether extract. Flavonoids were absent in petroleum ether and chloroform extract.

Antioxidant assay: Radical scavenging activities have huge importance due to the deleterious role of free radicals in biological systems.In certain conditions, over production of oxidants can cause imbalance leading to oxidative damage to large biomolecules such as lipids, DNA and proteins.Adverse side effects are present for many synthetic drugs even thou they protect against oxidative damage. Data

from both scientific reports and laboratory studies show that the plant contain a large variety of substance called "plant chemicals" or "phytochemicals" that possess antioxidant activity[16,17]. Studies have attributed that antioxidant properties are due to the presence of phenols and flavanoids[18]. Thus the presence of these components would have contributed to significant antioxidant activity of plant extracts. Antioxidant of phenolic compounds is based on their ability to donate hydrogen atom to free radicals[19]. The scavenging activity of a stable radical is considered a valid and easy assay to evaluate scavenging activity of natural compounds[20]. DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. From the present result it may be postulated that*Hibiscus surattensis*Linn reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles.The methanolic extracts exhibited high DPPH radical scavenging activity compared to other extracts in the present study.Superoxide anion is oxygen centered radical with certain selective reactivity. This species is created by a number of enzyme systems in auto-oxidation reactions and by non enzymatic electron transfers that univalently reduce molecular oxygen. It can also reduce certain iron complexes such as cytochrome[21]. The present study showed potent superoxide radical scavenging activity for *Hibiscus surattensis*Linn. Methanolic extract showed potent superoxide radical scavenging activity with IC_{50} value compared to standard ascorbic acid. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity[22]. One of the potent reactive oxygen species in the biological system is hydroxyl radical.It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and cause damage to cell[23]. The present study shows that the extracts had significant scavenging effects on hydroxyl radical, which increased with the increase in concentration from 10-160µg/ml.

CONCLUSION

Plants have been an excellent source of medicine since ancient times. This study reveals the phytochemical and anti-oxidant activity of aerial parts of *Hibiscus surattensis* Linn. The results show that the aerial parts of *Hibiscus surattensis* Linn have significant antioxidant activity. All the extracts showed a dose dependent DPPH scavenging activity of which the MEH showed 94.49% radical scavenging ability at 64µg/ml whereas at the same concentration other extracts like EAEH and CEH showed 88.49% and60.12% inhibition respectively, while PEH did not show 50% radical scavenging activity even at the concentration of 64µg/ml. Hydroxylradical scavenging activity was measured by assessing the inhibition of deoxyribose degradation by hydroxyl radicals. The MEH (IC_{50}=48.87±0.104) showed the maximum quenching ability. The scavenging effect of extracts on the superoxide anion radical decreased in the order MEH (IC_{50}=45.06±0.106) > EAEH (IC_{50}=77.31±0.146) > CEH (IC_{50}= 126.32±0.074). However further investigations on the isolation of active compounds present in the extracts and *in-vivo* studies are necessary to identify a potential chemical entity for clinical use.

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