Research Article

Pharmacognostical, Phytochemical and *In vitro* Anti- Oxidant Activities of Various Extracts of Flowers of *Hibiscus Rosa sinensis Linn*., (*Malvaceae*)

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ABSTRACT

The present aim of this study is to conclude the traditional approaches of the ancient healers about the plant *H.Rosa sinensis* belonging to the family Malvaceae. The flowers of *H.Rosa sinensis* was taken subjected to pharmacognostical, physiochemical, phytochemical analysis and anti-oxidant activity. A morphological study of flowers shows the presence of various diagnostic characters. Ash value, extractive value and moisture content were determined by quality standard of drug. The flowers extract shows the presence of various Phytoconstituent of flavonoids, tannin and protein etc. Anti-oxidant activity was done using various methods to protect against free radicals and therefore essential in obtaining and preserving the good health. The extract exhibited a concentration dependent scavenging activity.

Keywords: Herbal medicines, H. *Rosa sinensis,* Anti-oxidant activity.

INTRODUCTION

H.rosa sinensis (Malvaceae) which is widely distributed throughout the world. *H.rosa sinensis* is a perennial shrub. The approach of *H.rosa sinensis* is equally significant in alternative system of medicine as well as in conventional system of medicine[1]. As a traditional medicine, the fresh juice of the wild flowers is used to treat gonorrhea, the powdered roots are used to treat menorrhagia[2]. The alcoholic extract of flowers of *H.Sabdariffa* inhibited angiotensin-I converting enzyme[3]. The alcoholic extract of flowers of *H.rosa sinensis* has been proved to possess anticonvulsant property[4]. Powdered leaves of *H.rosa sinensis* lower blood pressure [5].

The infusion of petals is used as a refrigerant drink in fever. *H.rosa sinensis (Malvaceae)* is an ornamental plant often planted as a fence or hedge plant. The flowers have been reported to possess anti-implantation and anti spermatogenic activities. The extracts of *H.rosa sinensis* have also been shown a protective effect against the tumour promotion stage of cancer development. The leaves and flowers are observed to be promoters of hair growth and aid in healing of ulcer. Aerial part of *H.rosa sinensis* has calcium channel blocking action.

MATERIALS AND METHODS

Collection of flowers

H.rosa sinensis flowers containing five petals are collected, authenticated, cleaned and then they are shadow dried for 2 days and they are made into powder for further studies.

Preparation of extract

The powdered plant material is measured and they are taken in a conical flask. Following this the solvents such as petroleum ether, chloroform, ethyl acetate, alcohol and water were added to each flask then they are allowed to stand for 48 hours except for water. Finally, various extracts were obtained and they are subjected to further Phytochemical studies.

Macroscopic characters

The fresh plant was taken for various macroscopic organoleptic evaluations like colour, odour, size, shape, taste.[6]

Microscopic characters

Qualitative microscopic evaluation was carried out by taking transverse sections of fresh flowers of *H.rosa sinensis*. The thinnest section was selected and cleared by boiling with chloral hydrate solution for 20minutes and then carefully stained with phloroglucinol and Hydrochloric acid (1:1). Then mounted on a slide and a cover slip was placed over it and observed the different histological characters.[7]

Physicochemical parameters

The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, swelling index, foaming index, moisture content, ash value, pH were calculated as per Indian Pharmacopoeia.[8-13]

Preliminary Phytochemical screening

For preliminary Phytochemical screening, 100 g of powder drug was extracted with Distilled water. The mother extract obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various Phytoconstituent like glycosides, tannins, phytosterols, fixed oils and fats, proteins and amino acids, flavonoids, saponins, gums and mucilage etc. [14-17]

Invitro Anti-Oxidant Activity of flowers of *H.rosa sinensis*

Anti-oxidants obtained from plant are of greater benefit in comparison to synthetic one. It protects against free radicals and they are therefore essential in obtaining and preserving good health. Numerous works done on anti-oxidant activity of plant extracts are published. In this work anti-oxidant activity are given in unit's % mg, mg/ml or mg/g,that characterizes anti-oxidant content in studied extracts.

Hydrogen Peroxide Radical Scavenging Activity Assay

The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of Ruch. A solution of hydrogen peroxide (40millimetre) is prepared in phosphate buffer (50 millimetre, pH 7.4) and 2 millilitre of the solution is added to 1millilitre extract (1:20 dilution). The absorbance at 230nanometer is determined after 10 minutes. Hydrogen peroxide radical scavenging activity was expressed in terms of ascorbic acid equivalent (milligram/gram).[18, 19]

H_2O_2 + Cu +or Fe ^{2}+ $\xrightarrow{Cu^{2+} \text{or } Fe^{3+}}$ +OH +OH-

Reducing power assay

The reducing power was determined by the method of Athukorala. 1.0 ml extract was mixed with 2.5 millilitre of phosphate buffer (200 millimetre, pH 6.6) and 2.5 millilitre of potassium ferricyanide (30 millimetre) and incubated at 50°C for 20 minutes. 2.5millilitre of trichloroacetic acid (600 millimetres) was added to the reaction mixture, centrifuged for 10 min at 3000 Rotations per minute. The upper layer of solution (2.5millilitre) was mixed with 2.5 millilitre of distilled water and 0.5 millilitre of Ferric chloride (6 millimetres) and absorbance was measured at 700 Nanometre. Reducing power was expressed in terms of standard ascorbic acid (milligram/gram).[20, 21]

RESULTS AND DISCUSSION

Macroscopy of flowers of *H.rosa sinensis*

The macroscopic character was always served as useful keys in faster and early identification of plant material and also serves as an important standardization parameter. The macroscopic features of Root of *H.rosa sinensis* are described here. The organoleptic evaluation is discussed in table no.1.

Table 1: Organoleptic Evaluation of flowers of *Hibiscus Rosa sinensis*

S. No.	Organoleptic Parameters	Result
1	Colour	Pink Colour
2	Odour	Odourless
3	Taste	Sweet
4	Appearance	Smooth

Microscopy of flowers of *H.rosa sinensis*

Groups of pits, scleriods, and uneven distribution of colour pigments in couple of fibres with needle shape. Calcium oxalate crystals, pigmented fragments with calcium oxalate. Lignified stone cells are in group cellular fragments have scleriods, calcium oxalate and stone cells. The transverse section of *H.rosa sinensis* flower at ovary. The parts are Epidermis, Ovary, and Mitochondria Placenta.

Figure 1: Microscopy of the flower of *H.rosa sinensis*

Physiochemical studies

The determination of physico-chemical parameter is important in determination of adulterants and improper handling of drugs. Table- 2 shows the result of various physico chemical parameter of powdered drug carried out using standard methods. Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Ash values used to determine quality and purity of crude drug. It indicates the presence of various impurities like carbonate, oxalate and silicate. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent.

Table 2: Physiochemical studies of *H.rosa sinensis*

S.NO	PARAMETERS	VALUES*% (W/W)
1	Moisture Content	7.76 %
2	**Ash Values**	
	Total ash	10.2 %
	Acid insoluble ash	5.7 %
	Water soluble ash	4.2 %
	Water insoluble ash	9.3 %
3	**Extractive values**	
	Petroleum ether extract	57 %
	Chloroform extract	45 %
	Ethyl acetate extract	48 %
	Alcohol extract	64 %
	Aqueous extract	33 %

Phytochemical Analysis of H.*rosa sinensis*

The powder drug with different chemical reagents shows different color when seen on naked eye. The different color observed shows presence of different type of phytoconstitues (Table No.3). The extract was subjected to different qualitative chemical tests (Table no.3). The presence of various Phytoconstituent was observed during the test. Theses test were carried out over the different extracts. The data obtained is specified in table no.3.

Table 3: Preliminary Phytochemical analysis of flowers extract of *H.rosa sinensis*

S.No	Test	Powdered Drug	Extracts				
			Pet Ether	Chloroform	Ethyl Acetate	Alcohol	Aqueous
1	**Test for sterols**						
	a. Salkowski's test	+	-	+	-	+	+
	b. Libermann-burchard's test	+	-	+	-	+	+
2	**Test for carbohydrate**						
	a.Molish's test	-	-	-	-	-	-
	b.Fehling's test	-	-	-	-	-	-
	c.Benedict's test	-	-	-	-	-	-
3	**Test for proteins**						
	a.Millon's test	-	-	-	-	-	-
	b.Biuret test	-	-	-	-	-	-
4	**Test or alkaloids**						
	a. Hager's reagent	+	-	+	+	-	-
	b. Dragendroff's reagent	+	-	+	+	-	-
	c. Wagner's reagent	+	-	+	+	-	-
5	**Test for saponins**	+	-	-	-	+	+
6	**Test for tannins ($Fecl_3$ test)**	+	-	-	-	+	+
7	**Test for volatile oils**	-	-	-	-	-	-
8	**Test for mucilage**	-	-	-	-	-	-
9	**Test for flavonoids**						
	a. Shinoda test	+	-	+	+	+	+
10	**Test for coumarin**						
	b. $Fecl_3$ test	+	-	-	-	+	+
11	**Test for wax**	-	+	-	-	-	-

\+ Positive - Negative

Table 4: Hydrogen peroxide activity of Ethanolic extract of *H.rosa sinensis*

S.NO	Concentration in µg/ Ml	Percentage inhibition by std ascorbic acid	Percentage inhibition by EEHRS
1	10	42.72±0.68	34.5±0.283
2	20	71.08±0.89	55.7±0.317
3	40	82.7±0.96	68.3±0.319
4	60	85.45±0.86	72.5±0.332
5	80	92.42±0.65	89.2±0.340

EEHRS -Ethanolic Extract of .*rosa sinensis*, * mean of three readings ± SEM

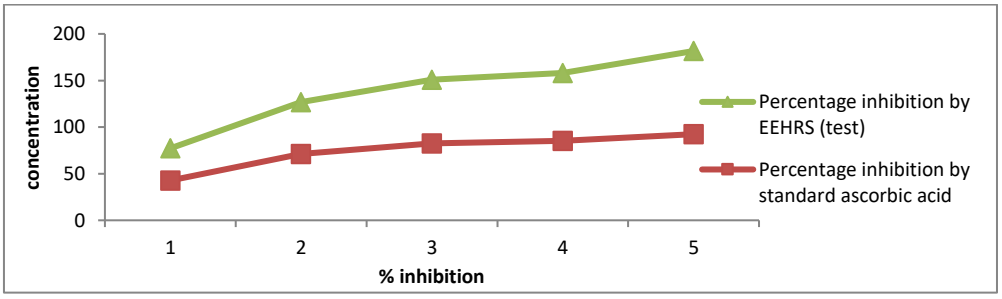

Figure 2: Hydrogen peroxide activity of Ethanolic extract of *H.rosa sinensis*

Reducing Power Assay

The reducing power of the compound may serve as a significant indicator of its potential antioxidant activity. Reducing power assay is used to evaluate the ability of natural antioxidant to donate electron. **Fig 3 s**hows the reducing power of the ethanolic extract of *H.rosa sinensis* compared to BHT as standard. The reducing power of the extract was found to be significant and dose dependent. The results obtained for the free radical scavenging activity against reducing power assay presented in **table 5.**

Table 5: Reducing power assay of ethanolic extract of *H.rosa sinensis*

Conc.in µg/ml	Percentage inhibition	
	Percentage inhibition by std ascorbic acid	Percentage inhibition by EEHRS
10	0.369±0.03	0.29±0.053
20	0.565±0.01	0.41±0.057
40	0.776±0.04	0.67±0.167
60	1.06±0.01	0.89±0.172
80	1.098±0.02	0.91±0.177

* mean of three readings ± SEM

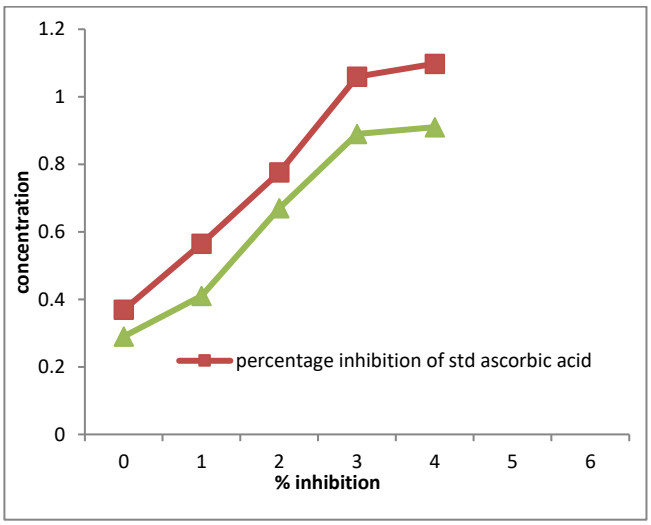

Figure 3: Reducing power assay of Ethanolic extract of *H.rosa sinensis* extract

DISCUSSION

Hence is selected for anti-oxidant activity. It possess more free radical scavenging activity than the other extract. But when compared with standard ascorbic acid the free radical scavenging activity is lesser done by hydrogen peroxide and reducing power assay method. Further studies are carried out in future.

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CONCLUSION

Thus Herbal plants containing their values in each and every part like root, stem, leaves, flowers and seeds are studied and made into herbal formulation for human intake to cure many diseases. Thus *H.rosa sinensis* as a medicinal plant also have more values. They should be studied further and further to bring out their medicinal values. Keeping this on considerance here it is made an attempt to make everyone know about the role of possessing Anti-oxidant activity and also done the microscopic, macroscopical studies.

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