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Research Article

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In vitro Phytochemical Analysis and Antioxidant Studies of Hibiscus Species

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ABSTRACT

Hibiscus is widely grown as an ornamental and medicinal plant. The Hibiscus rosa-sinensis leaves used as emollient, anodyne, and laxative. In the present study, phytochemical analysis, antioxidant assay of nine varieties of Hibiscus species were studied. The phytochemical analysis included tests for alkaloids, flavonoids, saponins, tannins, phenols, proteins, cardiac glycosides, terpenoids, carbohydrates and quinones using methanol and ethanol extracts of leaves. Tannins and Carbohydrates were present in all the samples taken for both the ethanol and methanol extracts, Proteins, Saponins and phenol were absent. Other phytochemicals were present in a few samples either in methanol or ethanol extracts. The antioxidant property of all extracts were analysed by DPPH free radical scavenging activity assay. The samples white with pink- many petals (WP) methanol extracts and yellow- 5 petals (Y) ethanol extracts showed the highest antioxidant activities with least IC_{50} values. Of these two solvents, methanol was found to be the best solvent to extract both, phytochemicals and antioxidants.

Keywords: *Hibiscus, Phytochemicals, Antioxidant, DPPH, Solvent extracts.*

INTRODUCTION

Hibiscus is a genus of flowering plant in the family Malvaceae, native to East Asia and it is known as rose mallow, Chinese hibiscus, China rose and shoe flower. *Hibiscus rosa-sinensis* is a bushy, evergreen shrub or small tree growing 2.5–5 m (8–16 ft) tall and 1.5–3 m (5–10 ft) wide, with glossy leaves and solitary, brilliant colour flowers in summer and autumn. It is widely grown as an ornamental and medicinal plant throughout the tropics and subtropics. Numerous varieties, cultivars, and hybrids are available, with flower colours ranging from white through yellow and orange to scarlet and shades of pink, with both single and double sets of petals. There are about 250 species of *Hibiscus* in the tropical and subtropical regions. Five species of *Hibiscus* are known to be endemic to Mauritius and these are namely *H. boryanus* D C, *H. columnaris* Cav., *H. fragilis* D C, *H. genevii* Bojerex Hook and *H. Ovalifolius.*

The detailed study of *Hibiscus rosa-sinensis* have been carried out worldwide which showed that Leaves are used as emollient, anodyne and laxative in Ayurveda[1]. In South Asian traditional medicine, various parts of the plant is used in the preparation of a variety of foods[2]. The flowers have been reported in the ancient Indian medicinal literature to have beneficial effects in heart diseases, mainly in ischemic disease and used in folklore medicine as emollient, refrigerant, aphrodisiac, brain tonic and cardio tonic. A decoction of flowers is also useful in bronchial catarrh, Menorrhagia, and fertility control[3, 4]. The extracts have also been shown to have hair growth potential[5], anticonvulsive activity[6] and hypoglycaemic activity[7]. Medicinal plants play a vital role for the development of new drugs. The extracts of *Hibiscus rosa-sinensis* and the crude drug itself are being used as an anti-ulcer, aphrodisiac, menorrhagic, oral contraceptive, laxative, anti-fertility, anti-implantation, abortifacient, epilepsy, leprosy, bronchial catarrh and diabetes. The plant was extracted with different solvents and the extracts were subjected to different qualitative chemical tests. The presence of various phyto constituents were observed when tested for glycosides, alkaloids, tannins, flavonoids, saponin and carbohydrates.

On the other hand, the ancient Indian medicinal literature reported that the flowers of *Hibiscus rosa-sinensis* have beneficial effects in heart diseases, mainly in myocardial ischemic disease, due to its enhancement of the myocardial endogenous antioxidants by an adaptative response and without producing any cytotoxic effects[8]. Recently it has been suggested that *Hibiscus rosa-sinensis* had a protective role against age and scopolamine- induced amnesia, indicating its utility in management of cognitive disorders[9].

MATERIALS AND METHODS

Collection of Samples

Different varieties of Hibiscus plant leaves were collected from different nurseries in and around Bangalore. The collection of leaves was based on colour of the flower and number of petals. The colour and number of petals of the flowers were Red- many petals, Red- 5 petals, pink- 5 petals, yellow with pink- 5 petals, yellow- 5 petals, white with pink- many petals, white- 5 petals, orange with pink- many petals, orange- many petals etc. Matured leaves of a single plant for all nine colours were collected separately. The leaves were kept in shade for drying.

Preparation of leaves extract

The collected leaves were shade dried at room temperature. The dried leaves were used for phytochemical analysis. The dried leaves of all nine varieties were powdered separately and stored for further analysis. 5g of the powdered leaf sample was taken and sample 50 ml of methanol and 50 ml of ethanol was added separately. The flasks were kept for solvent extraction at room temperature for 48 hours. After 48 hours, the methanol and ethanol extracts were filtered and reduced using rotary evaporator and the concentrated extracts were stored at room temperature for further use.

PHYTOCHEMICAL analysis

The preliminary phytochemical analysis was used to analyse the presence of compounds namely Alkaloids, Flavonoids, Saponins, Tannins, Phenols, Proteins, Cardiac glycosides, Terpenoids, Carbohydrate and Quinones. Using the below mentioned protocols.

ANTIOXIDANT analysis

The antioxidant assay was carried out to determine the antioxidant compounds present in the given sample. The DPPH free radical scavenging activity assay which is one of the widely used methods for quantitative analysis of antioxidant was used in the present study.

DPPH free radical scavenging activity assay

Dilutions of L-ascorbic acid were taken in the following concentration range (50, 100, 150, 200 and 250µg/ml) in each test tubes and make up the volume up to 1 ml using solvent. Then 3 ml of 0.1 mM DPPH was added into each test tube. The mixture was shaken well and incubated at room temperature for 30 minutes at dark place and absorbance was measured at 517 nm in spectrophotometer against solvent as blank and Solvent with DPPH (0.1mM) as control. The experiments were performed for all methanol and ethanol extracts. The scavenging activity was calculated from control sample OD using the following equation.

$$\% \text{ of radical scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Finally, IC50 value was calculated for each sample extract where dilutions of L-Ascorbic acid were used as standards. High IC50 value indicates less antioxidant capacity.

RESULTS

Phytochemical analysis

Phytochemicals are the chemical compounds present naturally in the plants. Some of the phytochemicals are Alkaloids, Flavonoids, Saponins, phenol, Tannins, Protein, Carbohydrate, Cardiac glycosides, Quinone, Terpenoids and Steroids. Composition of phytochemicals varied from plant to plant. In the present study the sample varieties showed the presence of various phytochemical compounds (Table-1a, 1b). The results showed that most of the phytochemicals are present in the selected varieties of *Hibiscus species* and methanol showed better extraction of phytochemicals when compared to ethanol. Tannins and Carbohydrates were present in all the samples taken for both the ethanol and methanol extracts, Proteins, Saponins and phenol were absent. Other phytochemicals were present in a few samples either in methanol or ethanol extracts.

Table-1a: Phytochemical analysis of *Hibiscus rosa- sinensis*

S.No.	Chemical constituents/ Hibiscus varieties extract		Alkaloids	Flavonoids	Saponins	Phenols	Tannins
1.	Red - many petals (R1)	Methanol	+	-	-	-	+
		Ethanol	+	-	-	-	+
2.	Red - 5 petals (R2)	Methanol	+	-	-	-	+
		Ethanol	-	-	-	-	+
3.	Pink- 5 petals	Methanol	+	-	-	-	+
		Ethanol	-	-	-	-	+
4.	Yellow with pink- 5 petals	Methanol	+	-	-	-	+
		Ethanol	-	-	-	-	+
5.	Yellow- 5 petals	Methanol	+	+	-	-	+
		Ethanol	-	-	-	-	+
6.	White with pink- many petals	Methanol	+	+	-	-	+
		Ethanol	-	+	-	-	+
7.	White- 5 petals	Methanol	+	-	-	-	+
		Ethanol	-	-	-	-	+
8.	Orange with pink- many petals	Methanol	+	-	-	-	+
		Ethanol	-	-	-	-	+
9.	Orange- many petals	Methanol	+	-	-	-	+
		Ethanol	-	-	-	-	+

Table-1b: Phytochemical analysis of *Hibiscus rosa- sinensis*

S.No.	Chemical constituents/ Hibiscus varieties extract		Proteins	Cardiac glycosides	Terpenoids	Carbo hydrates	Quinones
1.	Red - many petals (R1)	Methanol	-	-		+	-
		Ethanol	-	+	-	+	-
2.	Red- 5 petals	Methanol	-	-	-	-	-
		Ethanol	-	-	-	+	-
3.	Pink- 5 petals	Methanol	-	-	+	+	-
		Ethanol	-	-	-	+	-
4.	Yellow with pink- 5 petals	Methanol	-	+	-	+	-
		Ethanol	-	+	-	+	-
5.	Yellow- 5 petals	Methanol	-	+	+	+	+
		Ethanol	-	+	+	+	+
6.	White with pink- many petals	Methanol	-	-	-	+	+
		Ethanol	-	+	-	+	+
7.	White- 5 petals	Methanol	-	-	+	-	-
		Ethanol	-	-	-	+	-
8.	Orange with pink- many petals	Methanol	-	+	+	+	-
		Ethanol	-	-	+	+	-
9.	Orange-many petals	Methanol	-	+	+	-	-
		Ethanol	-	+	-	+	-

Antioxidant Activity

Antioxidant analysis by DPPH free radical scavenging activity assay was carried out to determine the % Antioxidant Activity of the methanol and ethanol extracts of all samples.

DPPH free radical scavenging activity assay

Absorbance of the methanol extracts and ethanol extracts of different concentrations with DPPH were measured at 517nm.

Fig.-1a: DPPH scavenging assay for methanol extracts

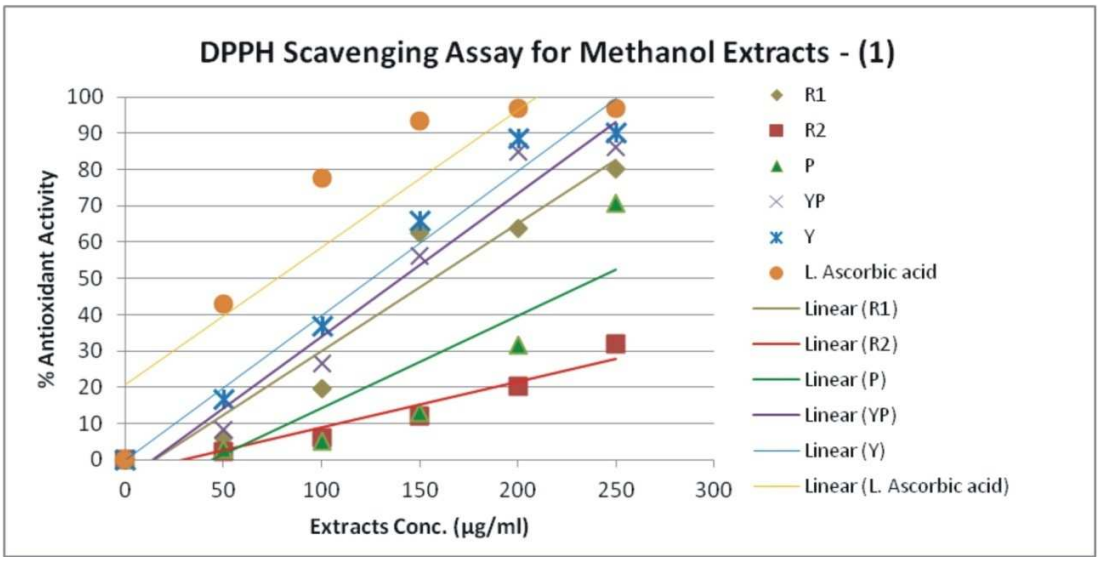


Fig.1b: DPPH scavenging assay for methanol extracts

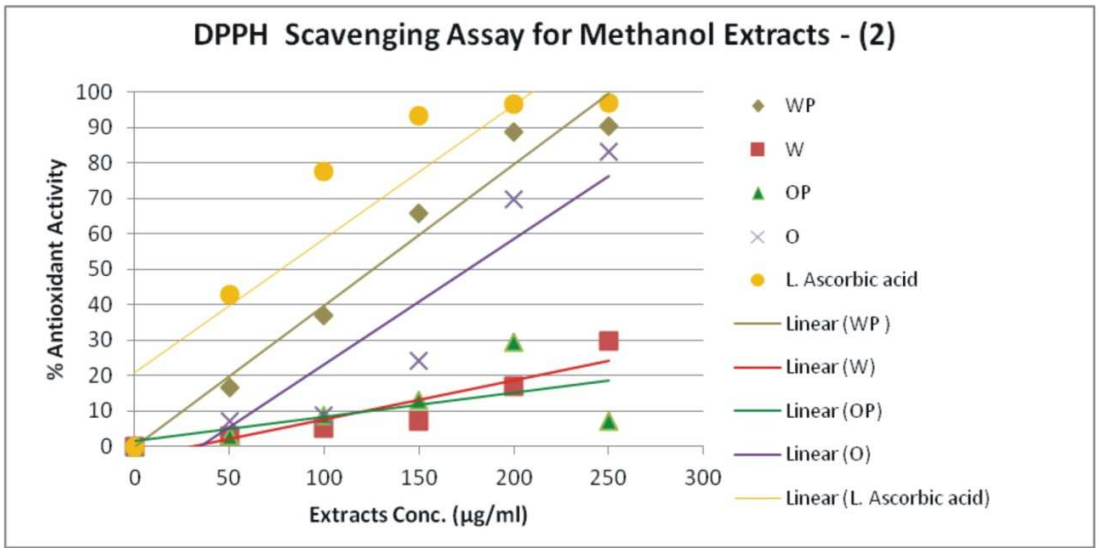


Figure-2a: DPPH scavenging assay for ethanol extracts

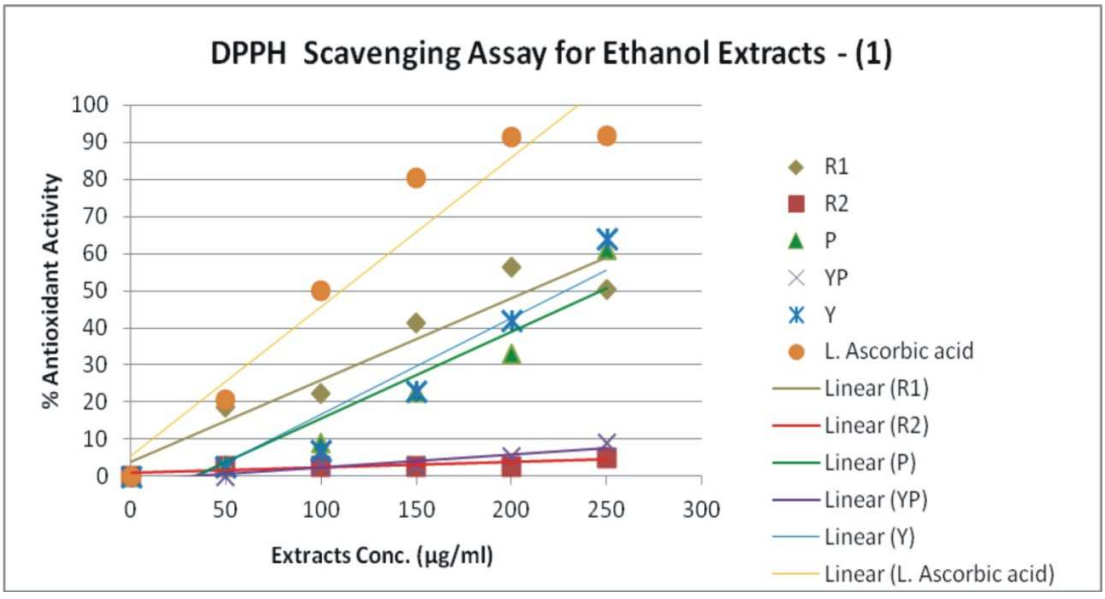
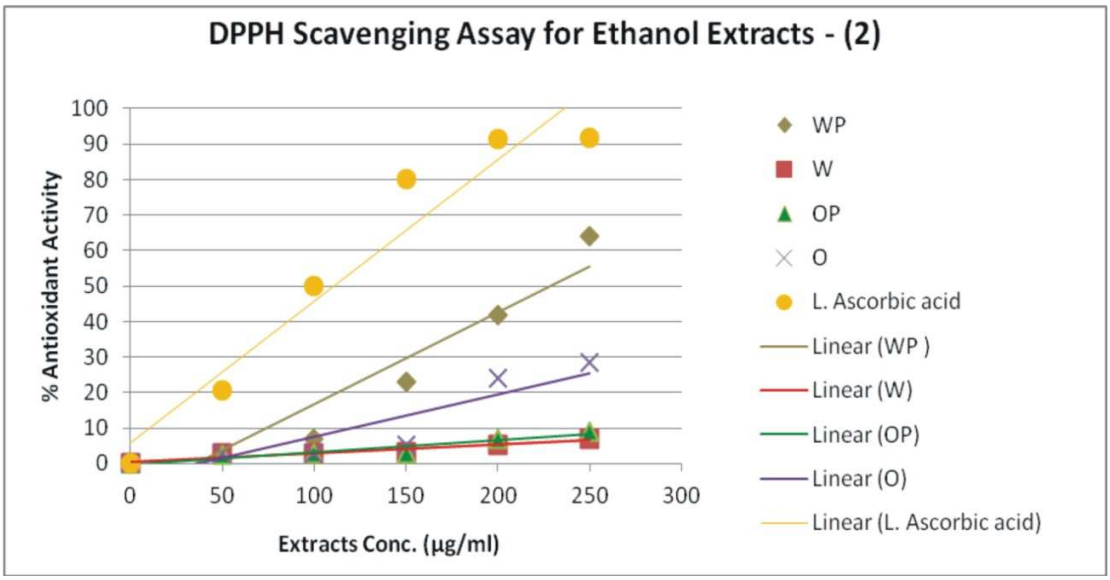


Figure-2b: DPPH scavenging assay for ethanol extracts



IC_{50} means concentration of sample (antioxidant) required for scavenging DPPH radicals by 50%. The value was calculated with reference to 50% Scavenging activity of L-Ascorbic Acid.

Table-2 : Antioxidant activity of samples compared to L. ascorbic acid as standards in terms of IC_{50} value

Samples	IC_{50} value of Extracts (mg/ml)	
	Methanol	Ethanol
Red - many petals (R1)	0.25	0.29
Red – 5 petals (R2)	0.68	4.49
Pink- 5 petals (P)	0.36	0.32
Yellow with pink- 5 petals (YP)	0.22	1.94
Yellow- 5 petals (Y)	0.21	0.19
White with pink- many petals (WP)	0.20	0.29
White- 5 petals (W)	0.77	2.71
Orange with pink- many petals (OP)	1.17	2.02
Orange- many petals (O)	0.26	0.60

The IC_{50} values decreases when antioxidant activity increases in the samples. The samples white with pink- many petals (WP) methanol extracts and yellow- 5 petals (Y) ethanol extracts showed the highest antioxidant activities with least IC_{50} values. The samples orange with pink- many petals (OP) methanol extracts and red- 5 petals (R2) showed least antioxidant activity as compared to other extracts.

DISCUSSION

In this present study, nine different varieties of *Hibiscus species* were selected. The extracts of sample were prepared by using methanol and ethanol as a solvent. The phytochemical test showed the presence of different phytochemicals in both, methanol and ethanol extracts. Alkaloids, Tannins and Carbohydrates was present in high amount in almost all extracts whereas Terpenoids, Flavonoids, Cardiac glycosides and Quinones were present in few extracts. Proteins, Saponins and phenols were not found in any of the solvent extracts. Among the two solvent extracts, the methanol was proved to the best solvent to extract maximum phytochemicals from the leaves of *Hibiscus rosa- sinensis* as compared to the ethanol.

The % antioxidant activity in *Hibiscus rosa- sinensis* was calculated by using DPPH free radical scavenging activity assay. The result showed methanol extracts of White with pink- many petals (WP) and ethanol extract of Yellow- 5 petals (P) has highest antioxidant capacity.

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