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ORIGINAL ARTICLE **OPEN ACCESS**

Exploring phytochemical potential and reconnoitering Total phenolic, flavanoid as well as antioxidant value of *Hibiscus sabdariffa*

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ABSTRACT

This Article deals with the phytochemical screening andin-vitro total flavonoids and phenolic contents in methanolic extracts of Hibiscus sabdariffa. H. sabdariffais also known as Lal-Ambari and to the family Malvaceae. It is widely useful for the treatment of many diseases, such as cough, dyspepsia, fever, hangover, respiratory tract infections, cold, hypertension and malaria. The screening of different solvents extracts like water, methanol, ethyl acetate and petroleum ether shows that leaves of H. sabdariffa contain various medicinally important phytochemicals, namely, alkaloids, flavonoids, glycosides, sterols, tannins and triterpenes. In contrast, anthraquinones, coumarins, resins and saponins are absent in leaves of H. sabdariffa. The UV visible spectroscopy technique, involving aluminum chloride & Folin-Ciocalteau methods respectively for total flavonoids & total phenols contents was used for analyses. Quercetin was used as standard compound for total flavonoids and tannic acid for total phenols. The total flavonoid content (16.36±0.88 mg QE/g) has been found in methanolic leaf extract of H. sabdariffa whereas the total phenolic content (48.44±10.46 mg TAE/g) was found in leaf extract of H. Sabdariffa. The extracts from H. sabdariffa were also evaluated for their antioxidant potential by evaluating their DPPH radical scavenging activity. The findings suggest that aqueous extract had the best %scavenging potential of 73.30% which was near to the ascorbic acid with 77.23% scavenging. Methanolic extract showed 71.38% scavenging while the ethyl acetate and petroleum ether extracts had lower scavenging potential of up to 36.38% and 36.06% respectively. The results of this study reveal that leaves of H. sabdariffa plant have great potential for phytochemicals that can be used as pharmaceuticals of the new generation.

Key Words: *Hibiscus sabdariffa, phytochemical screening, total flavonoids, total phenols, Folin-Ciocalteau.*

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INTRODUCTION

Hibiscus Sabdariffa plant, commonly known as Roselle (English)or Lal Ambari (Hindi), is found in tropical and sub-tropical regions in the world [1]. The plant belongs to the family Malvaceae and is an annual or perennial, erect, woody-based sub-shrub with a deep root system [2,3]. Different parts of the plant including the leaves, seeds, stem and calyces are used traditionally for many purposes, such as flavoring agent, hot and cold beverages and herbal beverage [4]. Beverages, sourced from the calyces of *H. sabdariffa,* have been used traditionally for the treatment of many diseases, such as cough, dyspepsia, fever, hangover, respiratory tract infections, cold, hypertension and malaria [5].

Phytochemicals are bioactive compounds in plants. They are naturally synthesized in all parts of the plant such as leaf, stem,root, flower, seed etc. *H.sabdariffa* have been reported to contain numerous phytochemicals that include alkaloids, flavonoids, saponins, tannins and others [6,7].The active phyto-constituents of *H. sabdariffa* have been used for the treatment of gastro-intestinal disorders, liver diseases, fever, hypertension some cancers and other diseases [8,9] .Flavonoids are the largest group of naturally occurring phenolic compounds. They are found to have many biologicalactivities including antioxidant, antimicrobial, anticancer and antiulcer [10]. Flavonoids and phenols are abundant in most plants, and the bioactivity of the some of these compounds have been identified to be beneficial to human health [11,12]. The presence of a phytochemical of interest may lead to its further isolation, purification and characterization. Then, it can be used as the basis for a new pharmaceutical product. The aim of the present investigation was to identify and quantify potent bioactive compounds (phytochemicals) of *H. sabdariffa*.

MATERIAL AND METHODS

Study was conducted at the department of P.G. Studies & Research in Biological Science, R.D.University, Jabalpur, India during the period from March 2018 to 2021.

Collection and identification of plant material

The plant of *Hibiscus Sabdariffa* was collected from the Rani Durgavati University, campus, Jabalpur. It was identifiedwith the help of literature [12, 4] and authenticated by a plant taxonomist at the herbarium of the State Forest Research Institute (SFRI). Jabalpur, (India).

Preparation of plant extracts

The freshly collected leaves of *Hibiscus sabdariffa* were dried under shade for 7 to 10 days until brittle and dried to a constant weight. After drying, leaves were ground in a household grinder and the powder obtained was sieved through 100µM test sieve. The remaining coarse powder, that could not be sieved, was again ground and sieved as stated above. Now, the sieved powder material was collected in an air tight jar for future use.

The leaves powder was extracted using the cold percolation method as described by Harborne [13] Extraction procedure involved solvents with their decreasing polarity, i.e.,water, methanol, ethyl acetate and petroleum ether by using soxhlet extractor (JSGW, India).

Phytochemical Screening

The qualitative screening of phytochemical was done according to standard procedures [13, 14]. Phytochemical screening was done to check the presence of primary metabolites such as – carbohydrates, lipids and proteins and secondary metabolites like alkaloids, flavonoids, sterols,triterpenes and glucosides etc. Testing of different phytochemical.

Quantitative analysis

The total flavonoids content (TFC) as described by Olajuyigbe *et al*., [15] and total phenolic content (TPC) as described by Wu *et al*., [16] were determined.

Statistical analysis

Mean, standard deviation, regression and correlation of the triplicate data were calculated using the Microsoft Excel.

Total flavonoid content

Determination of total flavonoids content is based on aluminum chloride method [15]. In this test, 10mg of quercetin was dissolved in 10Mlof methanol and various aliquots of 100-500 µg/Ml were prepared in methanol. One gm of dried extract was dissolved in 100ml of methanol, filtered and made up to the 100 ml. One Ml(1mg/Ml) of this extract was used for the estimation of flavonoids. 1Ml of 2% $AlCl_3$ methanolic solution was added to 3Ml of extract and standard and allowed to stand for 60 minutes at room temperature followed by measurement of absorbance at 420nm using a UV-Vis spectrophotometer (Systronics, India).

Total phenolic content

The total phenolic content was quantified using the modify Folin- Ciocalteau method [16]. 100mg tannic acid was dissolved in 100 ml methanol. Various aliquots of 100-500µg/Ml were prepared in methanol. 1 g of dried extract was dissolved in 100 ml methanol. 0.3 Ml of this extract (equivalent to 150 mg dry weight) was for the estimation of phenol. 0.3 ml of extract or standard was mixed with 1.5ml of Folin-Ciocalteau reagent followed by 1.2 mL of 75% (w/v) sodium carbonate solution. The mixture were vortexed for 15 seconds and allowed to stand for 30 minutes at room temperature. The absorbance was measured at 765nm using U.V. Spectrophotometer (Systronics, India).

Determination of Antioxidant Property

The antioxidant activity of leaf extracts of *Hibiscus sabdariffa* was estimated by their ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The method used was as described previously with some modification [17]. For the study 60 µM DPPH solution was used and the all the sample extracts were used in concentration range of 20-100µg/ml. The extracts, 500 µl was mixed with the equal volume of DPPH solution and allowed to stand for 1 h at room temperature in dark. The absorbance of reaction mixture was measured at 517 nm using a spectrophotometer. The ascorbic acid in the same concentration was used as positive control. Optical density of freshly prepared DPPH solution was taken as control. The absorbance was taken three times, each after 30 min. The average value was used for estimation of %scavenging potential by the given formula;

$$\% \text{ scavenging for DPPH radical} = \frac{A - Ax}{A} \times 100$$

where, A- Absorbance of control (DPPH solution with ethanol)
Ax- Absorbance of sample (DPPH solution with test solution)

The antioxidant potential of extracts from *Hibiscus sabdariffa* was also estimated by a Thin layer chromatography method suggested by Sreenivasan *et al*., [18]. Where the sample extracts were loaded on a TLC plate (5x20 cm) prepared with silica gel G mixed with 2% $CaSO_4$ as binder. The plates were allowed to dry at RT then activation was done at 110°C for 30 min. For separation of compounds methanol and chloroform (85:15 v/v) was used as the mobile phase. After separation the plates were sprayed uniformly with 0.04% DPPH solution in methanol using a spray gun for 5 sec. The image was observed under visible light exactly 2 min after spraying with the help of a white light illuminator. The area of bright yellow bands against the purple background showed the position of antioxidant band having DPPH radical scavenging activity.

RESULTSAND DISCUSSION:

Phytochemical screening

The results of the qualitative phytochemical screening of *H. Sabdariffa* leaf extract with different solvents are summarized in Table 1.

Primary metabolites like carbohydrate, proteins and lipids along with 7 secondary metabolites (Alkaloids, Cardiac Glucosides, Flavonoids, phenols. Tannins, Sterols, Triterpenes) were detected and the 4 secondary metabolites (Anthraquinones, Coumarins, Resins, Saponins) were not detected in the leaf extract of *H. sabdariffa.*In the present investigation, carbohydrate, protein and lipids were detected in the *Sabdariffa* leaf. Alkaloids are found to be better extracted by water and methanol and petroleum ether. Flavonoids were found to be extractable in only methanol.Carbohydrate, lipids and tannins were found to be extractable in all the solvent system such as water, methanol, ethyl acetate and petroleum ether. Sterols and triterpenes were extracted in water and methanol. Cardiac glycosides were extractable only in water. While saponins, resins, coumarins and anthraquinones were not detected.

Table 1: Phytochemical screening of different solvent ofleaf extract of *Hibiscus sabdariffa*

Phytochemical	Tests	Aqueous	Methanol	Ethyl acetate	Petroleum ether
Alkaloids	Dragendroff test	+	+	-	+
	Mayer's test	-	-	-	-
Anthraquinones	-	-	-	-	-
Carbohydrate	Molisch's test	+	-	-	+
	Benedict's test	-	-	-	-
	Fehling's test	+	+	+	+
Cardiac Glucosides	Keller-Killiani	+	-	-	-
Coumarins	-	-	-	-	-
Flavonoids	-	-	+	-	-
Lipid	Solubility test	-	-	+	+
	Sudan III	+	+	+	+
Protein	Biuret test	-	+	-	-
	Xanthoprotic	-	+	-	-
Phenolic	-	+	+	-	-
Resins	-	-	-	-	-
Saponins	Foam test	-	-	-	-
Sterols	Salkowski's test	+	+	-	-
Tannins	Gelatin test	-	-	-	+
	Ferric chloride test	-	-	-	-
	Lead acetate	+	+	+	+
Triterpenes	-	+	+	+	-

(+) Indicates presence and (-) indicates absence of phytochemicals.

Estimation of total flavonoids content

Quercetin used as standard compound. The calibration curve for quercetin usingabsorbance at 420 nm in different concentration is presented in Figure 1. It is the based on the prediction equation Y= 0.003 X-0.042, R^2=0.995, The mean value of total flavonoids content in methanol extract of *H. sabdariffa*leaf in terms of quercetin equivalent was 16.36±0.88 mgQE/g respectively.(Table-2)

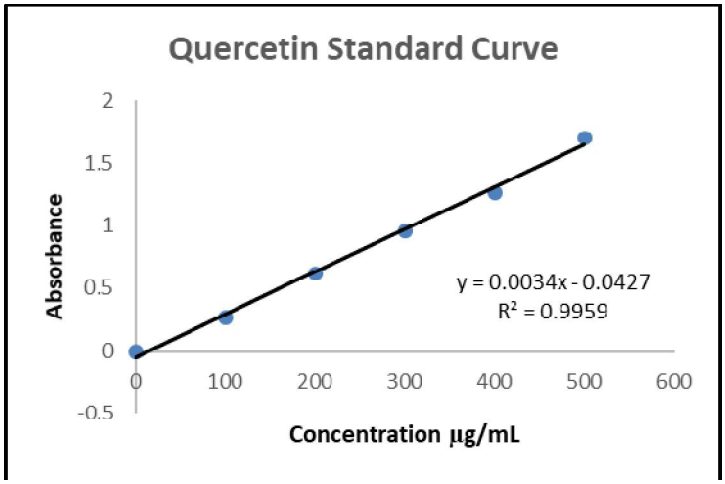

Figure 1 : Standard curve of quercetin using absorbance at 420nm.

Estimation of total phenolic content

Tannic acid was used as standard compound. The calibration curve for tannic acid using absorbance at 765 nm in different concentration is presented in Figure 2. It is the based on the prediction equation Y=0.003 X-0.040, R^2=0.096, Where Y is absorbance at 765nm and Xis tannic acid equivalent. The mean value of total phenolic content of methanol extracts of *H. sabdariffa* leaf in term of tannic acid equivalent were 48.44±10.46 mg/g tannic acid equivalent respectively (Table-2).

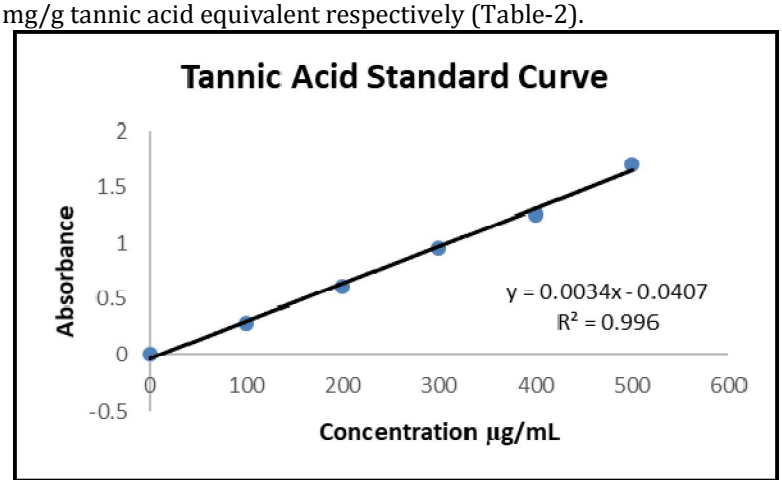

Figure 2: Standard curve of tannic acid using absorbance at 765nm.

Table No. 2. Total flavonoids and phenolic content in methanolic extract of *H. Sabdariffa*

Phytochemical	I.	II.	III.	Mean±S.D.
Total flavonoids content (mg QE/g)	16.75	15.36	16.98	16.36±0.88
Total phenolic content(mg TAE/g)	43.75	60.42	41.15	48.44±10.46

S.D = Standard deviation

The antioxidant activity as estimated by DPPH radical scavenging potential showed that at 100 µg ml^{-1} concentration, the aqueous extract of *H. sabdariffa* had the highest %scavenging of 73.30%. While the methanolic extract, ethyl acetate extract, and petroleum ether extract could scavenge 71.38%, 36.38%, and 36.06% of DPPH free radicals respectively. The results compared with the positive control, ascorbic acid with 77.23% scavenging potential (Figure 2). The aqueous extract had the best antioxidant potential among all the extracts (Table 1) and compared to ascorbic acid it had good potential to be used as an antioxidant agent. The findings of TLC analysis showed that the yellow spot of antioxidant compound that appeared after reaction with DPPH was seen only for the aqueous and methanolic extracts and not in ethyl acetate and petroleum ether extract.

Table No. 3. Antioxidant potential of different extract of *H. Sabdariffa* against the DPPH radicals

Concentration (µg/ml)	% scavenging of DPPPH radicals				
	Aqueous	Methanol	Ethyl acetate	Petroleum ether	Ascorbic acid
20	45.53	33.62	20.85	18.94	26.81
40	51.91	34.89	23.94	20.43	38.51
60	60.74	51.28	27.13	28.83	51.28
80	69.36	66.17	33.62	33.51	65.53
100	73.30	71.38	36.38	36.06	77.23

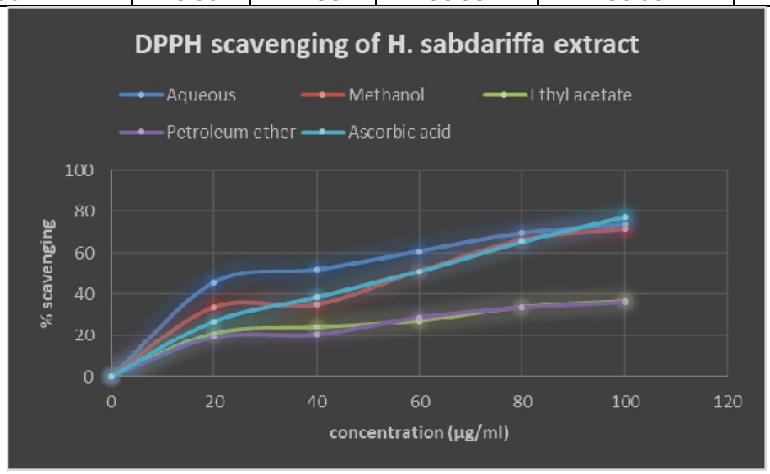

Figure 3: Comparison of antioxidant potential in extracts of *H. sabdariffa* with the ascorbic acid against DPPH radicals

CONCLUSION

The phytochemical screening of the different solvents of leaf extracts of *H. sabdariffa* showed the presence of alkaloids, flavonoids, phenolic, tannins, sterols, triterpenes and others. which have great medicinal and pharmacological properties. The extract also show strong antioxidant activity which may be due to presence of high flavonoids and phenol content. The findings of the present study suggest that *H. sabdariffa* could be a potential source of natural antioxidant. Further investigations are required to isolate and characterize the active constituents from this plant to evaluate their therapeutic role.

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