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HPTLC-Bioautography guided identification of antioxidant and antimicrobial activity of Hibiscus rostellatus

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Abstract— Hibiscus rostellatus belonging to family, malvaceae,is a herb ,used in traditional medicine for the treatment of inflammation and renal diseases. Methanolic extract of Hibiscus rostellatus was fractionated using different solvents of increasing polarity like petroleum ether, ethylacetate, methanol and water. The preliminary phytochemical screening showed the presence of carbohydrates, steroids, flavonoids, tannin. Methanolic extract of hibiscus rostellatus roots were screened for their antimicrobial, antioxidant activity. The test microorganisms included bacteria of Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa. It was concluded that Hibiscus rostellatus root extract was well effective against all these microorganisms. HPTLC-DPPH assays were used to identify the antioxidant activity of plant extract. This activity was estimated by using the mobile phase Chloroform:EthylAcetate:Acetic Acid (4.6:3.1:2.3 v/v/v). There was a successful chromatogram developed and detection of a band in the sample was observed. Later, the band showing antioxidant characteristic property was identified by DPPH Assay.

Key words: HPTLC- Bioautography, Antimicrobial, Antioxidant, DPPH, Hibiscus rostellatus

INTRODUCTION

Hibiscus rostellatus Guill. & Perr. (syn. Hibiscus furcatus, Hibiscus hispidissimus belonging to the family malvaceae commonly known as wild hibiscus [1]. The plant is a large climber having reddish stems that are covered with hooked prickles (4-8). The leaves are alternately arranged, 6- 8cm, palmately 3-5 lobed, hairy and heart shaped at the base. Leaf margins are toothed, lobes are long pointed, leaf stalks 5- 10cm long and prickly. Stipules are lane shaped. Yellow flowers arise singly from leaf axils which are carried on 3-5cm long prickly stalks. 8-12 bracts below the flowers with leafy appendages. Hairy sepals. Seed capsules are 1cm long, ovoid, pointed, enclosed in enlarged sepal cup. This plant is commonly found in the evergreen forests of Western Ghats. The flowering period is November-January. It is distributed throughout India. Hibiscus has a long history of traditional use for various health purposes. Leaves and flower contain hibiscus acid, garcinia acid, hibiscatin, gassyptrin, malic acid, tartaric acid etc. which shows antioxidant properties. This plant also contains alkaloids, anthocyanin, and quercetin which shows medicinal properties [2-3]. The tribal healers of kerala region use this plant to treat liver disease as it improves digestion as plant has antihelminitic

and anti-inflammatory activity. The juice of the leaves are mixed with honey and used in treatment of eye diseases. Decoction of the root bark is used as remedy for poisons, swellings and cleansing the kidneys [4-6]. However, there is no clarity regarding the main active compounds in root responsible for its medicinal properties. In recent years, thin layer chromatography-bioautography method has been widely and efficiently used for the screening and quantification of antioxidant and antimicrobial compounds. Therefore, present study was planned to study the preliminary phytochemical detection and identification of antioxidant and antimicrobial components in the root of hibiscus rostellatus using 2,2-diphenyl-1-picryl-hydrazyl (DPPH)- HPTLC bioautographic method [11].

MATERIALS AND METHODS

Plant material

The roots of Hibiscus rostellatus was collected from Ismail Yusuf college campus area, Jogeshwari, Mumbai district, Maharashtra in October 2019. The plant was authenticated from blatter herbarium of St. Xavier college, Mumbai, Maharashtra, India. The root of the plant was shaded dried, powdered and passed through 70 mm mesh sieve and stored in an airtight container for further use.

Preparation of extract

The shade dried flower powder, about 1.0 g, was weighted and sonicated with 10 ml of methanol at room temperature for 1 hr. the extract was filtered through whattman no.1 filter paper then extract was stored at 4°C for further use.

Proximate analysis

Determination of extractive values, loss on drying, total ash value, water soluble ash value and acid-insoluble ash value were performed by using standard procedure [8]. Data were presented in table no.1

Preliminary phytochemical screening

Preliminary phytochemical studies of various solvent extracts of H. rostellatus root were carried out by performing qualitative chemical test as per standard procedure [9]. The results were mention in table no.1

Estimation of phytoconstituents

Estimation of total alkaloid, total phenol, total flavonoid and total tannin contents were carried out by standard procedures [7]. Results were noted.

DPPH-HPTLC Bioautographic Assay

Chromatography was performed on 10 x 4 cm HPTLC plates coated with silica gel 60 F_{254} (Merk HX94930454). Samples (5µl) were applied as a band using Linomat 5 applicator (CAMAG, Switzerland). The plates were developed to a distance of 70 mm in an twin trough developing chamber (CAMAG) with Chloroform:Ethyl acetate:Acetic Acid (4.6:3.1:2.3 v/v/v) as developing solvent. After development, the dried HPTLC plate was immersed in 0.03% (m/v)

DPPH methanolic solution. After derivatization, the plates were air-dried and scanned out under 254 nm and white light using a CAMAG Scanner 3.

Radical Scavenging Assay

Radical scavenging activity of root extract against DPPH was determined by using standard procedure [10]. The antioxidant compound, which donate hydrogen to reduce DPPH. The change in colour (from deep violet to light yellow) was measured at 517 nm on a UV visible light spectrophotometer. Radical scavenging activity was calculated by the following formula.

% Inhibition = $[(A_0 – A_1)/ A_0] \times 100$

A_0 = Absorbance of blank sample

A_1 = Absorbance of test extract sample

HPTLC-Antimicrobial Bioautographic Assay

The HPTLC-bioautographic method was employed for the determination of antibacterial activities of the methanolic root extract of hibiscus rostellatus. The extract was applied on 10 x 4 cm HPTLC silica gel 60 F_{254} (Merk HX94930454) plates using Linomat 5 applicator (CAMAG, Switzerland). The plates were developed in Twin trough chamber containing respective mobile phases. The developed plate was dried and to obtain well separated bands. The developed silica plates were placed into sterile petri plates. The culture of Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa which were grown at 37°C at 24 hours. was mixed with Mueller & Hinton agar medium and poured onto the sterile petri plate containing developed silica gel plate such that it will form a layer over the TLC plates. The plate was incubated at 37^0C for over 24 hours. After incubation the plates were observed by pouring INT (2-(-4-iodophenyl-)-3-(-4-nitrophenyl)-5-phenyl-2H-tetrazolium) dye in sterile conditions and further incubation of plates for 20 mins. For location and visualization of antibacterial substances, tetrazolium salts are usually used, which are converted by the dehydrogenases of living microorganisms to intensely colored formazan. After the treatment with INT dye the clear inhibition zones of active components will appear on the plate in comparison to the colored background.

RESULT AND DISCUSSION

Preliminary phytochemical screening

Different solvents may yield extracts with varying compositions and properties hence various solvents like ethyl acetate, petroleum ether, methanol and water were used to prepare extracts of H. rostellatus. In present study the extractive values of petroleum ether, ethyl acetate, methanol and aqueous were found to be 0.8%, 2.4%, 7.0% and 6.6% w/w respectively. Preliminary phytochemical screening of various extracts of H. rostellatus root reveals the presence of alkaloids, carbohydrates, amino acids, flavonoids, triterpenoid, saponin, tannin. Amongst the various solvent methanol extract showed the presence of most of the secondary metabolites (Table no.1). the screening of phytoconstituents of H. rostellatus root extract was performed on silica gel 60 F_{254} HPTLC plates. The Rf values of separated components were 0.006, 0.124, 0.458, 0.665, 0.735, 0.848, 0.952 (Fig. 2). possible components present in

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methanolic extracts were seen by TLC followed with exposure to iodine, dragondroff reagent, p-anisaldehyde-sulfuric acid and 10% ferric chloride (Fig.3).

Phytochemical Components	Chemical Test	Petroleum Ether	Ethyl Acetate	Distilled Water	Methanol
Alkaloids	Mayer's reagent	-	-	-	+
	Wagners reagent	-	-	-	-
Amino acids	Milon's test	-	-	-	+
	Ninhydrine test	-	-	-	-
Carbohydrates	Molisch test	+	+	-	+
	Barfoed's test	-	-	-	-
Flavonoids	Shinoda	-	-	-	+
	Alkaline reagent	+	+	+	+
	Zinc hydrochloride	-	-	-	-
Cardiac glycosides	Killer killiani	-	+	-	-
	Legal's test/ Caumarin	+	+	+	-
Proteins	Biuret	-	-	-	-
Steroids & triterpenoids	Libermann-Burchard	-	+	+	+
	Salkowski	-	+	+	+
Saponin	Froth	-	-	+	-
Tannin	FeCl3	-	+	-	+
	Lead acetate	+	+	+	+

Table 1. Phytochemical screening of different extracts of H. rostellatus root

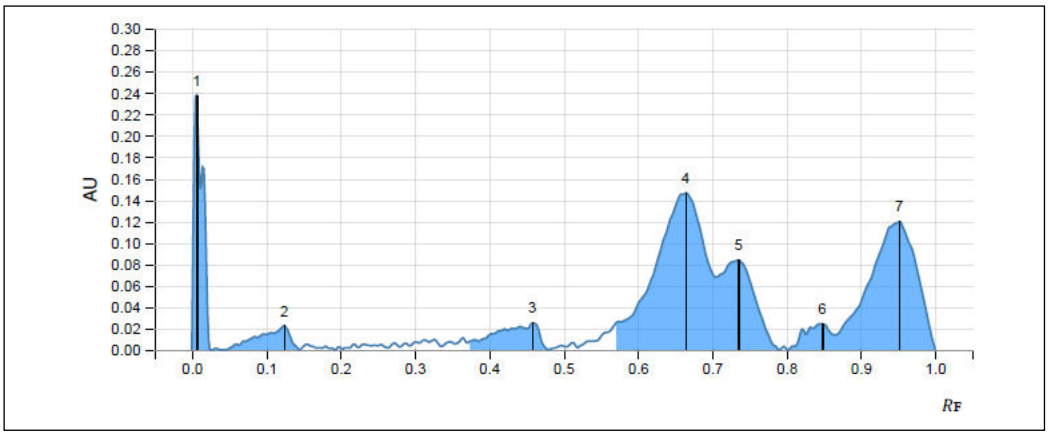

Fig. 1 HPTLC chromatograph of methanolic extract of H. rostellatus root

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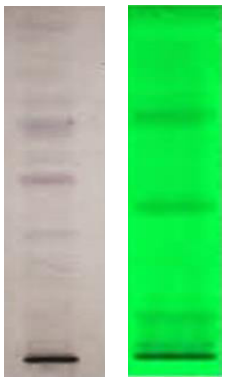

Fig. 2 HPTLC images of methanolic extract before derivatization

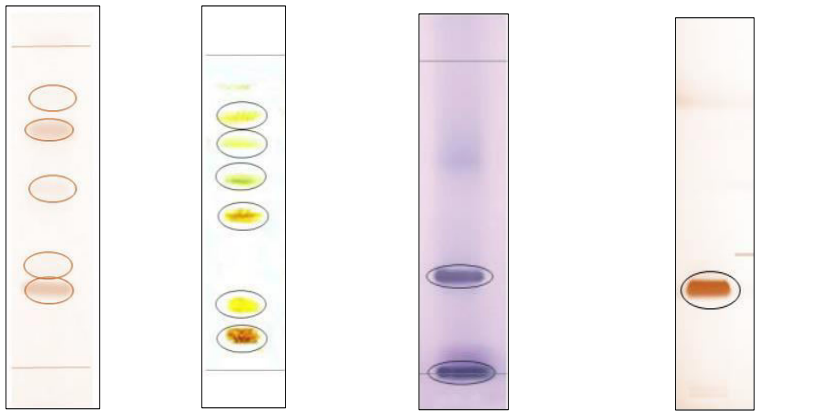

Fig. 3 HPTLC images of methanolic extract after derivatization

Physico-chemical parameters

Physico-chemical parameters including moisture content, total ash content, acid insoluble ash, water soluble ash was found to be 7.36%, 4.33%, 5.63 % and 21.49% (W/W) respectively (Table no. 2). Total alkaloid content was measured by using standard atropine in methanolic extract and expressed in terms of atropine equivalent as mg/g of extract (the standard curve equation: $Y = 0.0052x + 0.024$, $R^2 = 0.9846$) (Fig. 4) the concentration of alkaloid was 0.41 mg/g. The total phenolic content was examined using the Folin-ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $Y = 0.0056x + 1.5556$, $R^2 = 0.9138$). the concentration of total phenolic content was measured 10.12 mg/g (Fig. 5). Total tannin contents were examined using the Folin-ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $Y = 0.1124x + 0.068$, $R^2 = 0.9939$). the concentration of tannin was measured 0.453 mg/g in extract (Fig. 6). The concentration of flavonoids in methanolic extract of H. rostellatus was determined using spectrophotometric

method with aluminium chloride. The content of flavonoids was expressed in terms of quercetin equivalent (the standard curve equation: Y = 0.0064x + 0.1184, R^2 = 0.9797). the concentration of flavonoids in methanol extract was 1.84 mg/g (Fig. 7).

Sr. No.	Physico-chemical parameters	Values (gm%)
1	Moisture Content	7.36
2	Total Ash	4.33
3	Water soluble ash	21.49
4	Acid insoluble ash	5.63

Table no. 2 The Physico-chemical analysis of H. rostellatus root

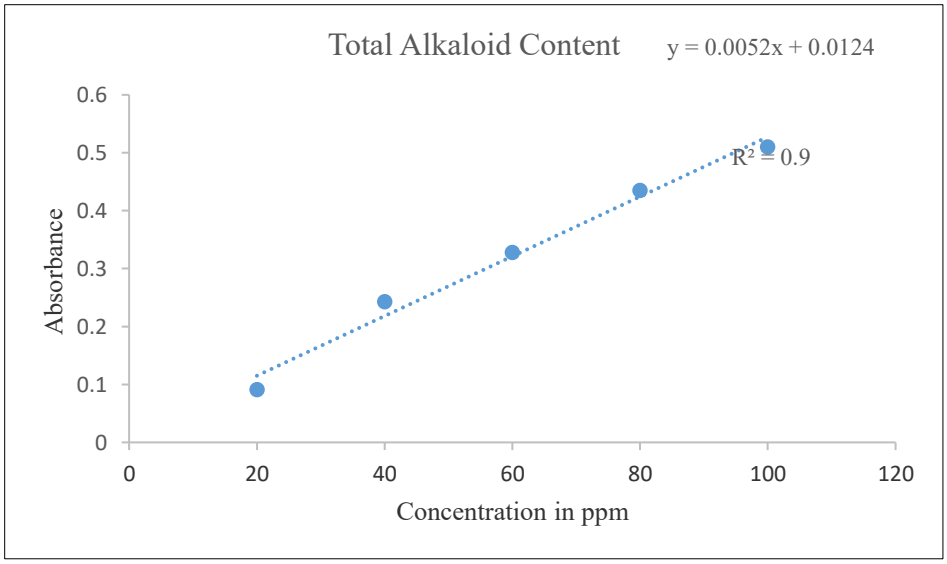

Fig. 4 Calibration curve of total alkaloid content

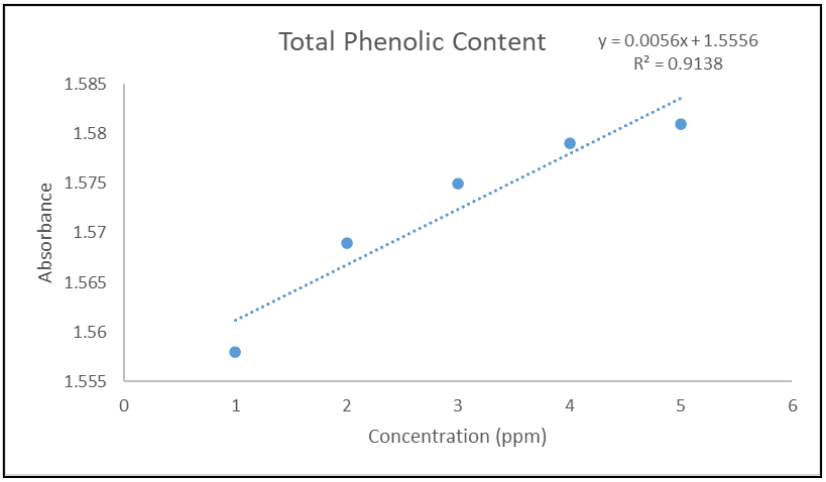

Fig. 5 Calibration of total phenolic content

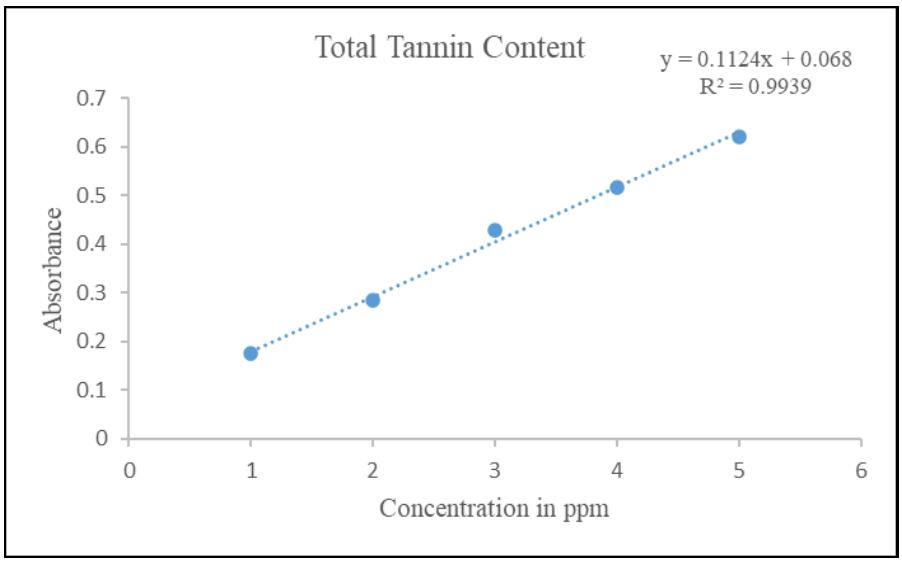

Fig. 6 Calibration curve of total tannin content

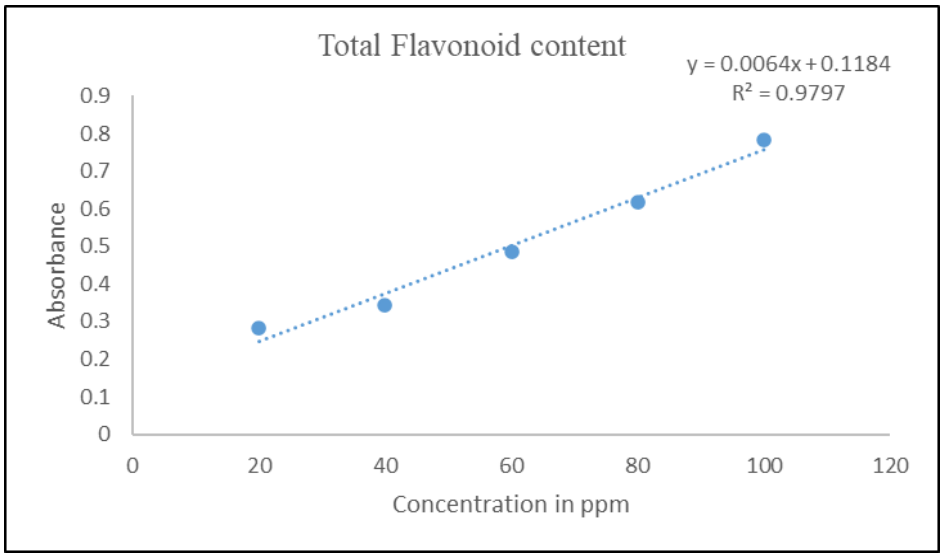

Fig. 7 Calibration of total flavonoid content

Antioxidant assay

The methanolic root extract was found to have antioxidant molecules which has been evidenced through the HPTLC-bioautography analysis for antioxidants (Fig. 8a). The Rf value of antioxidants compounds are 0.455, 0.671, 0.750 and 0.952 (Fig. 8b). DPPH free radical scavenging activity exhibited scavenging potential of methanolic flower extract of H. rostellatus was 19.04 %.

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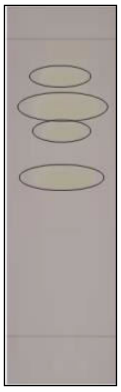

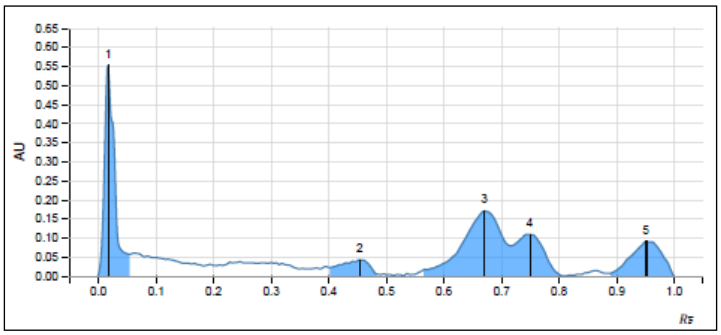

Fig. 8a HPTL images of DPPH assay

Fig. 8b HPTLC chromoagraph of DPPH assay

Antimicrobial assay

Root extract of H. rostellatus shown zone of inhibition against Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa (Fig. 9). The Rf values of antimicrobial active compound was 0.750.

The bioautography showed that the compounds responsible for antioxidant activity and antimicrobial activity could be alkaloids (Fig.1). the alkaloids of H. rostellatus with retention factor of 0.750 shows both antioxidant and antimicrobial properties although alkaloids and other organic compounds with retention factor 0.455, 0.671 and 0.952 shows only antioxidant activity.

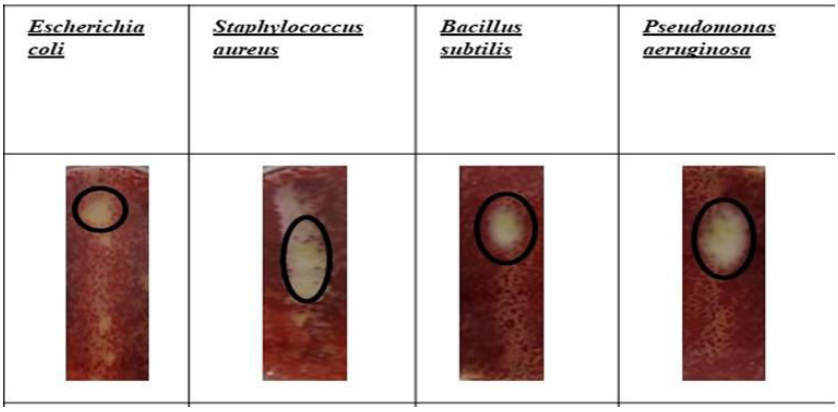

Fig. 9 HPTLC images of antimicrobial assay

CONCLUSION

The root of H. rostellatus was proven to have both antioxidant and antimicrobial activity which has been confirmed through HPTLC guided identification. These findings will be useful towards further isolation of alkaloids and other organic compounds from methanolic extract of H. rostellatus root.

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