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Research Paper :

HPTLC- aided phytochemical fingerprint analysis as a tool for evaluation and antiviral activity using HeLa cell cultures of *Bauhinia variegata* plant

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Bauhinia variegata was investigated for preliminary phytochemical analysis and characterization by various instrumental techniques. Acetone extracts of *Bauhinia variegata* was very good antibacterial activity and also minimum inhibitory concentrating of different virus using HEL cell cultures, HeLa cell cultures, and Vero cell cultures but MIC of Herpes simplex - 1 and 2, vaccinia virus, vesicular stomatitis and Herpes simplecx-1 (TK ACV^{I}) were observed very good antiviral activity of *Bauhinia variegata* DMSO extracts and good minimum cytotoxic concentration activity.

Key words : *Bauhinia variegata,* Antiviral activity, Analysis, Phytochemical screening

Bauhinia variegata of caesalpiniaceae family is widely used in vitiated conditions of *Pitta* and *Kapha,* diarrhoea, dysentery, skin diseases leprosy, intestinal worms, tumors, wounds, ulcers, inflammations, scrofula, proctoptosis, haemorrhoids, haemoptysis, cough, menorrhagia and diabetes. The roots and bark are astringent, cooling, constipating, depurative, anthelminitic, vulnerary, anti-inflammatory and styptic.

Antimicrobial activity of seeds proteins of *Bauhinia variegata*[1]. Antimicrobial efficacy of leaves extract of *Bauhinia variegata in vitro* condition[2]. Trypsin inhibitors from *Bauhinia variegata* seeds[3]. Anthocynins in *Bauhinia variegata* flowers of West Bengal[4]. Presence of some flavonoids, amino acids, sterols, triterpenoids and anthoynins have been reported from different parts of *Bauhinia variegata* plants[5-9]. Two new long chain compunds from *Bauhinia variegata* leaves has been reported[10].

Pharmacological activity of Bauhinia variegata:

Anti oxidant activity and phenolic contains of Asian vegetables of *Bauhinia variegata* and other *Bauhinia species*[11]. *Bauhinia variegata* Linn. (caesealpiniaceae), wood decidous tree has snake-antivenom, antifungal, antitumor, anti-inflammatory and aphrodisiac properties[12].

MATERIALS AND METHODS

Experimental:

The following instrument was used for HP-TLC Screening

– Linowate 5 semi auto application with CAL-MAG software.

– HP-TLC plate: silica gel 60 F_{254} (merck).

– Antioxidant activities of different extracts was recorded on *in vitro* models. Purchased from Himedia ltd. Antiviral activities was done by Rega institute of medical research, Belgium.

– The extract screening for antimicrobial activity on different types of gram + ve and gram – ve strains.

– UV visible spectra are recorded on uv-visible spectro photometer.

Plant materials :

The *Bauhinia variegata* Leaves were collected from the plant growing from patola farmhouse, patan during January month.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Extraction :

Bauhinia variegata leaves collected from patola farmhouse from Patan. The leaves were dried at RT for 45 days. After cursed it. The leaves powder extract in acetone on Soxhlet apparatus for 48 hrs. After collect acetone layer and solvent evaporate under reduced pressure.

Preliminary phytochemical screening :

Phytochemical screening of ABV was carried out for the presence of phenol, tannins, steroids, alkaloids, anthraquinones and flavanoids.

Instrumental analysis :

20 grams of the drug was extracted with acetone (3 x 50 ml) and extracts was cooled and the solvent was removed by heating the etracts on a water bath maintained at 60^0 C. stock soluation (2 mg/ml) on the extract was prepared in acetone and was applied in precoated TLC plate. The plate was dried at room tempreture than devloped in following solvent system. Solvent system Cyclo haxene. Ethyl acetate (7:3 v/v). After devolpement the plate was dried at room tempreture and scanned at UV 254 nm and chromatograms were recorded.

Electroric spectra and gas chromotogratly:

Electroic spectra of Bauhinia Variegata leaves:

In the present work electronic spectra of *Bauhinia Variegata* leaves extract were carried out which showed ëmax value at 254 nm which indicated blue shift (n -p*) trasition indicate x = o functional group present in componds.

Gas chromatography pharmaceutical application:

GC analysis of *Bauhinia Variegata* leaves confirm that consist 5 component which gives the peak at 12.42, 13.24, 14.06, 20.98, 21.56 and 22.76 min of time, respectively and found with the help of different type of pytochemical studies suggest tannins, anthraquinones, phenols, steroids, alkaloids and flavanoids are presence in *Bauhinia variegata* plant.

Pharmacological evalution

Antioxidant activity :

Various disease conditions are associated with free radicals scavengers are well known for their therapeutic activity. A number of anti-oxidant like ascorbic acid, pyrogallol, vitamin E, curcumins etc have been shown to effectively quench these radicals and hence were found to be very beneficial in prophylaxis of the above mentioned disease. There are three types of free radicals, which cause disease conditions in humans. They are antiradical, super oxide scavenging and nitric oxide scavenging activities.

Antiradical activity :

Antiradical activity is measured by decrease in absorbance at 516 nm, of methanolic solution at colored DPPH[13-14]. Decrease in absorbance in the presence of test compound at different concentration was measured after 15 minutes. The EC_{50} is the concentration of the test solution that can bring about 50% decrease in absorbance. In this study pyrogallol was used as a reference standard. The anti radical activity of the test compound are showed in Table 1.

Table 1 : Atiradical activity of *Bauhinia variegata* observed with DPPH

Compound	Concentration µg/ml	% Inhibition	EC_{50} (µg/ml)
Standard	1.0, 1.2, 1.4,	22, 30, 36,	1.7 µg/ml
Pyrogallol	1.6, 1.8, 2.0	44, 54, 62	
Bauhinia	20	13.33	57.0 µg/ml
variegata	30	21.11	
	40	28.88	
	50	43.33	
	60	47.77	
	70	64.44	
	80	72.22	

Super-oxide anion activity of Bauhinia variegata plant:

Superoxide radicals generated in riboflavin light NBT System was measured[15]. The reaction mixture contains 50mM Phosphate buffer pH 7.6, 20 µg riboflavin, 12 mM EDTA and NBT 0.1 mg/3 ml. added in that sequence. The reaction was started by illuminating the reaction mixture with different concentration of the test solution started reaction, that absorbance was measured at 590 nm and EC_{50} was calculated. Ascorbic acid was used as standard antioxidant. The super oxide anion activity of *Bauhinia variegata* observed with riboflavin light NBT System showed in Table 2.

Table 2 : Super oxide anion activity of *Bauhinia variegata* observed with riboflavin light-NBT system

Sample	Concentration µg/ml	% Inhibition	EC_{50} (µg/ml)
Standard	5, 10, 15	24.06, 40.34,	12.5 µg/ml
Ascorbic	20, 25	58.04, 75.24,	
acid		92.55	
Bauhinia	2	21.27	
variegata	4	38.25	
	6	50.18	
	8	59.65	6.9 µg/ml
	10	70.81	
	12	85.00	
	14	90.49	

Nitric oxide scavenging activity :

Nitric oxide is implicated in inflammation, cancer and other pathological condition[16-17]. The procedure for nitric oxide scavenging activity is based on the principle the sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrites ions that can be estimated using 0.5 ml. Greiss reagent (1% N- (1-napthyl

ethylenediamine, dihydrochloride)[18]. The absorbance of the chromophore formed was read at 546 nm while using curcumin as positive control. The nitric oxide activity of *Bauhinia variegata* observed with Griess reagent. Showed in Table 3.

Table 3 : Nitric oxide activity of *Bauhinia variegata* observed with Griess reagent

Sample	Concentration µg/ml	% Inhibition	EC_{50} (µg/ml)
Standard curcumin	5, 10, 15 20, 25	37.56, 47.30, 58.33, 65.38, 77.30	11.2 µg/ml
Bauhinia variegata	10	23.46	
	15	35.10	
	20	43.46	24.4 µg/ml
	25	50.58	
	30	62.30	

Antiviral activity :

In antiviral activity live cell cultures were used. In this method cell was grown on solid media. After any cytopathic effect was checked with comparing uninoculated cell line. Various cell lines are use for the study of antiviral activity of drug e.g. HEL cell cultures, Hela cell cultures and Vero cell cultures etc.

The minimum concentration of extract which the viral inhibited or reduced virus induced cytopathogenicity by 50% is good for drug. There is a co-relationship between MIC (minimum inhibition concentration) and MCC (minimum cytotoxic concentration). Higher MCC and lower MIC indicate usefulness of drug.

Different viruses infect different cell lines :

For the result, MIC of different virus using HEL cell cultures. Here Brivudin, Ribavirin, Acyclivir and Ganciclovir are use as control. MIC of Herpes simplex-1 and 2, vaccinia virus, vasicular stomatitis and Herpes simplex-1 (TK ACV^{l}) were observed very good antiviral activity of *Bauhinia variegata* Dmso extract. *Bauhinia variegata* have been good antiviral agent because their MIC for all five viruses are less then 10 µg/ml and their MCC is less than 50 µg/ml the respective data are given in Table 4 :

Bauhinia variegata plant extracts for the antiviral activity viruses using Hela cell cultures. Here Brivudin, Ribavirin, Acyclovir, Ganciclovir are use as control MIC of vesicular stomatitis, coxsackie virus, Respiratory syncytial virus were very good antiviral activity of *Bauhinia variegata* DMSO extracts and good minimum cytotoxic concentration (MCC) activity. *Bauhinia variegata* have been good antiviral agent because their MIC for there viruses are less than 10 µg/ml. and their MCC is less than 50 µg/ml. The respective data are given Table 5.

Cytotoxicity and antiviral activity of vero celll cultures are negligible for *Bauhinia variegata* plant extract.

Table 4 : Cytotoxicity and antiviral activity in HEL cell cultures

Compounds	Minimum cytotoxic concentration[a] (µg/ml)	Minimum inhibitory concentration[b](µg/ml))				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccini Virus	Vesicular stomatits Virus	Herpes simplex virus-1 TK KOS ACV^{r}
Bauhinia variegata	50	>10	>10	>10	>10	>10
Brivudin (µM)	>250	0.08	0.8	6	>250	250
Ribavirin (µM)	>250	250	250	50	150	250
Acyclovir (µM)	>250	0.4	0.16	>250	>250	150
Gancicolvir (µM)	>100	0.032	0.096	>100	>100	4

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathologenicity by 50%

Table 5 : Cytotoxicity and antiviral activity in HeLa cell cultures

Compounds	Minimum cytotoxic concentration[a] (µg/ml)	Minimum inhibitory concentration[b](µg/ml))		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
Bauhinia variegata	>50	>10	>10	10
Brivudin (µM)	>250	250	>250	>250
(S)- DHPA(µM)	>250	150	>250	>250
Ribavirin(µM)	>250	30	150	10

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathologenicity by 50%.

Antibacterial activity :

Antibacterial activity was determined by the Agar cup plate method. petriplates containing 20 ml nutrient agar medium (pH 7.2-7.4) were seeded with 17 cultures of the becterial strains. After the study of *Bauhinia variegata* plant have also good antibacterial activities of different grams (on the basis of gram's reaction) shows different results. The extracts of *Bauhinia variegata*

Table 6 : Antibacterial activity minimal inhibition concentration

Sr. No.	Genus	Species	*Bauhinia Variegata*
1.	Proteus	Mirabilis	50 µg/ml
2.	Proteus	Vulgaris	50 µg/ml
3.	Staphylococcus	Aureus	50 µg/ml
4.	Micrococcus	Luteus	25 µg/ml
5.	Mycobacterium	Smegmatis	50 µg/ml
6.	Bacillus	Cereus	50 µg/ml
7.	Clostridium	Sporegenes	250 µg/ml
8.	Klebsiella	Pheumoniae	50 µg/ml
9.	Salmonella	Typhimurium	200 µg/ml
10.	Shigella	Flexneri	150 µg/ml
11.	Vibrio	parahaemolyticus	25 µg/ml
12.	Pseudomonas	Aeruginosa	200 µg/ml
13.	Bacillus	Pumilus	25 µg/ml
14.	Staphylococcus	Epidermidis	200 µg/ml
15.	Escherichia	Coli	50 µg/ml
16.	Escherichia	Coli	25 µg/ml
17.	Saccharomyces	Cerevisiae	50 µg/ml

show notable antifungal activity, which is tested against yeast *saccharomyces cerevisiae*, and also good activity against E coil, it is 25 mg/ml. for both organisms.

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