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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(4):324-332

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Anti viral in HEL cell, HeLa cell cultures, antibacterial and antioxidant activity of *Acacia arabica* seeds extracts by the use of DPPH free radical method

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ABSTRACT

Acacia arabica was investigated for preliminary phytochemical analysis and characterization by various instrumental techniques. Methanolic extracts of Acacia arabica seeds was very good antibacterial activity and also minimum inhibitory concentrating of different virus using HEL cell cultures HeLa cell cultures Vero cell cultures but MIC of Herpes simplex - 1 and 2, vaccinia virus, vesicular stomatitis and Herpes simplecx-1 (TK ACV[1]) were observed very good antiviral activity of Acacia arabica seeds DMSO extracts has good minimum cytotoxic concentration activity and also screening for various pharmaceutiocals activities. Such as anti oxidant and microbial activities.

Key Words : Phytochemical screening, *Acacia arabica* analysis, Anti viral, Anti oxidant activity.
Parts Use : Seeds, .

INTRODUCTION

The phytochemically screening of this medicinally useful plant in much less available. *Acacia arabica's* family name is Minosaceae, and English name is India Gum, *Arabica tree*. In Ayurvedic medicine, babul is considered a remedy that is helpful for treating premature ejaculation. *Acacia bar* is a powerful astringent. The barks from the branches yield 7-12 percent tannins and used for asthma, bronchitis, diabetes, dysentery, diarrhea and skin diseases. The gum was also applied to open wounds as an antiseptic balm. A decoction of the bark, mixed with rock salt should be used as a gargle in treating tonsillitis.

Proanthocynidins and other phenolic in *Acacia arabica* leaves of southern Africa [1] .Saponins and phenolic content in plant dietary additives of traditional subsistence community in Tanzania [2]. Chemical structures of compounds isolated from *Acacia arabica* and other *Acacia arabica* species also indexed of the heading catechin gallate derives from bark [3]. Pod phenolic of phytotoxicity of *Acacia arabica* [4]. Phytochem and biol activity of growing in Egypt [5]. ∝-Spinasterol of *Acacia arabica* [6]. Nutritive evaluation of some *acacia arabica* tree leaves from Kenya [7]. Method treatment of HIV assocd. Condition of *Acacia Arabica* [8]. Screening *in vitro* cultures of *Acacia arabica* plant for their antibiotics activity [9]. Tryptopham contents of total proteins, albumins and globulins in *Acacia Arabica* [10]. Antimicrobial activity of seeds proteins of *Acacia Arabica* [11]. *Acacia arabica* is also indexed at the heading phenolic compounds and tannins [12].

Pharmacological activity of *Acacia arabica*

Effect of *Acacia arabica* on blood glucose levels of normal and alloxan diabetic [13]. observation on blood pressure response to injection of *Acacia arabica* plant extracts in rats [14]. Commonly used India, the flowers of *Acacia arabica* appeared to their treatologic effects in rats [15].

EXPERIMENTAL SECTION

(A) 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 Hi media ltd. Antiviral activities was done by Rega institute for medical research, Belgium.
- The extract screening for antimicrobial activity on different types of gram + and gram – strains.
- UV visible spectra are recorded on uv-visible spectro photometer.

(B) Plant material :- The *Acacia arabica* seeds were collected from the plant growing in the forest of Dahod district during may month.

(C) Extraction :- *Acacia arabica* seeds collected from Dahod district Jungle. The seeds were dried at Rt for 60 days. After crushed it. The seeds powder extract in acetone on soxhlet apparatus for 48 hours. The acetone extract was subjected to preliminary phytochemical screening for the detection of major chemical group.

RESULTS AND DISCUSSION:

Preliminary phytochemical screening : Phytochemical screening of Acetone extracts *Acacia arabica* seeds was carried out for the presence of phenol, tannins, steroids, terpenoids, alkaloids and anthraquinones.

TLC finger print profile of acetone extract :

TLC firger print profile was developed for acetone extracts of *Acacia arabica* seeds using HP-TLC.

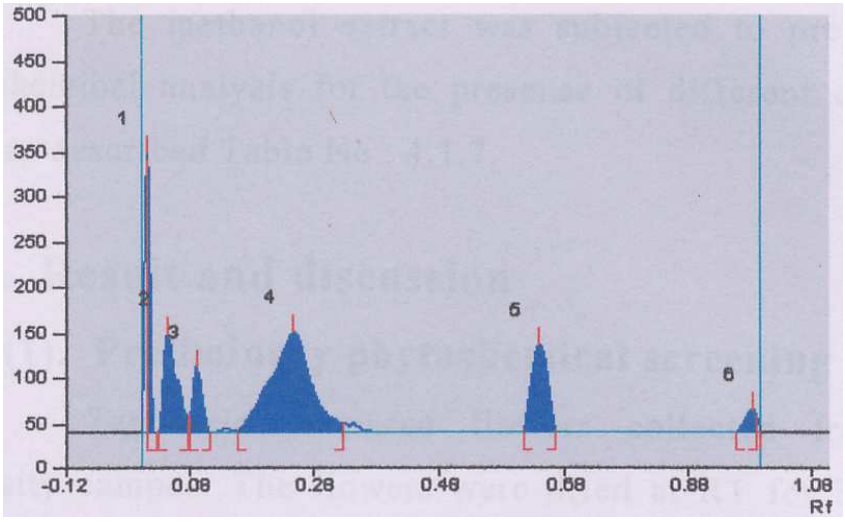
Solvent system : Benzene (10v/v)

TLC of acetone extract of Acacia Arabica showed bands in Uv 254 nm out of which four bands at Rf 0.01, 0.04, 0.06, 0.26, 0.66 were major. The Complete TLC Finger print profile including number of bands, their Rf relative percentage is given in table and absorption spectra of resolved bands in table – 1 and TLC chromatogram of successive acetone extract of *Acacia arabica' seeds* scanned at 254 nm in figure-1.

Table – 1: TLC finger print profile of acetone extract of *Acacia arabica* seeds

Peak	Start Rf	Max Rf	Hight %	Relative %
1	0.01	0.24	12.1	9.74
2	0.04	0.35	5.84	4.29
3	0.06	0.45	6.98	6.63
4	0.26	0.46	9.54	4.45
5	0.66	0.52	4.78	4.35
6	0.96	0.57	2.51	2.31

Figure 1 :TLC Chromatogram of acetone extract of *Acacia Arabica* seeds scanned at 254 nm



Pharmacological evaluation :-

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 [16-17]. 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-2.

Table – 2:Antiradical activity of pyrogallol observed with DPPH

Compound	Concentration µg/ml	% Inhibition	EC_{50} (µg/ml)
Standard Pyrogallol	1.0, 1.2, 1.4, 1.6, 1.8, 2.0	22, 30, 36, 44, 54, 62	1.7 µg/ml
Acacia arabica	30 40 50 60 70 80 90	25.55 32.22 37.55 45.55 55.55 65.55 84.44	62.00 µg/ml

Super-oxide anion activity of *Acacia arabica* seeds:-

Superoxide radical is known to be very harmful to the cellular components. Superoxide radicals generated in riboflavin light NBT System was measured [18]. 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 also test compound are showed in table-2.

Table –3:Super oxide anion activity of Ascorbic acid observed with riboflavin- light-NBT system

Sample	Concentration µg/ml	% Inhibition	EC_{50} (µg/ml)
Standard Ascorbic acid	5, 10, 15 20, 25	24.06, 40.34, 58.04, 75.24, 92.55	12.5 µg/ml
Acacia arabica	5 10 15 20 25	33.60 42.44 55.34 64.48 80.58	12.5 µg/ml

Nitric oxide scavenging activity :-

Nitric oxide is implicated in inflammation, cancer and other pathological condition [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). The absorbance of the chromophore formed was read at 546 nm while using curcumin as positive control [19]. The nitric oxide activity of *Randia dumetoram* observed with Griess reagent. Showed in table. No.3.

Table – 4 Nitric oxide activity of Curcumin 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
Acacia arabica	20 25 30 35 40	39.23 46.53 54.48 58.66 63.71	28.1 µg/ml

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.

For this reason the study of viruses are not possible like bacteria. The viral activity is checked by Cytopathic effect in which morphological change in particular cell line is observed. Normally, the drugs, which are nucleotide analogue, protein inhibitor, or drugs, which prevent the assembly of virus, are used.

Sample preparing :

For antiviral activity of herbal plants of *Acacia arabica* seeds was extracts in DMSO solvent and also extracts in water.

Different viruses infect different cell lines :

For the result, minimun inhibitory concentration of different virus using HEL cell cultures. Here Brivudin, Ribavirin, Acyclivir and Ganciclovir are used as control. MIC of Herpes simplex-1 and 2, vaccinia virus, vesicular stomatitis and Herpes simplex-1 (TK ACV^I) were observed very good antiviral activity of *Acacia arabica* seeds Dmso extracts and good minimum cytotoxic concentration activity. *Acacia arabica seeds* have been good antiviral agent because their MIC for all five viruses are less than 10mµg/ ml and their MCC is less than 50 mg/ml. The respective data are given in Table no- 5.

The result of differrent plant extracts For antiviral activity viruses using HeLa cell cultures. Here Brivudin, Ribavirin, Acyclovir, Ganciclovir are used as control. Minimum inhibitory concentration (MIC) of vesicular, stomatitis, Coxsackie virus, Respiratory syncytial virus were observed very good antiviral activity of *Acacia arabica* DMSO extracts and good minimum cytotoxic concentration (MCC) activity. *Acacia arabica* have been good antiviral agent because their MIC for all three viruses are less than 10 µg/ml and their MCC is less than 50 µg/ml. The data are given in Table no-6.

Table – 5 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
Acacia Arabica	50	>10	>10	>10	>10	>10
Acacia Arabica	>50	>50	>50	>50	>50	>50
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 - 6Cytotoxicity 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
Acacia Arabica	50	10	>10	>10
Acacia arabica	>50	>50	>50	50
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%

Table – 7 Cytotoxicity and antiviral activity in Vero cell cultures

Compounds	Minimum cytotoxic concentration[a] (µg/ml)	Minimum inhibitory concentration[b](µg/ml))				
		Para Influenza-3 Virus	Reo virus-1	Sindbis virus	Coxsacke virusB4	Punta Toro virus
Acacia arabica	>50	>50	>50	>50	>50	>50
Acacia arabica	>50	>50	>50	>50	>50	>50
Brivudin (µM)	>250	>250	>250	>250	>250	>250
(S)- DHPA(µM)	>250	>250	>250	>250	>250	>250
Ribavirin(µM)	>250	150	150	150	>250	50

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

Cytotoxicity and antiviral activity of Vero Cell cultures are negligible for *Acacia arabica* extracts. The data are given in Table no- 7.

Antibacterial activity :

The antimicrobial drugs occupy a unique was the vehicle in the history of medicine. The realization that certain microorganisms are successfully resisting the "wonder drugs" not only impels a lease less search for new systemic antimicrobial agents but also forced for sober return to certain ancillary art of the medical and surgical treatment of infectious disease.

Table– 8:Antibacterial activity of *Acacia Arabica* seeds extracts on different bacteria:

Treatment	Concentration µg/ml	Zone of inhibition (mm)			
		S. Aureus	***S. Epidermidis***	***P. Aeruginosa***	***K. Pneumoniae***
Methanolic extract	**150**	**18**	**23**	**24**	**25**
Acetone extract	**150**	**15**	**19**	**19**	**18**
Standard Ciprofloxacin	**2**	**29**	**30**	**30**	**30**

Table– 9:Antifungal activity of *Acacia Arabica* seeds extracts on different bacteria:

Treatment	Concentration µg/ml	Zone of inhibition (mm)	
		C.Albicans	***A.Niger***
Methanolic extract	**150**	**20**	**22**
Acetone extract	**150**	**19**	**16**
Standard Greseofulvin	**2**	**30**	**30**

Acknowledgement

The authors are greatful to the Deaprtment to the Chemistry Hemchandracharya North Gujarat University Patan for providing the necessary laboratory facilities. We are highly indebted to Director, C.D.R.I., Lucknow and Director, SICART, Vallabh Vidhyanagar, for all possible supports and the facilities to carry out this work.

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