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Antioxidant and Antimicrobial Properties of *Acacia catechu* Plant

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Abstract: The present study involves the checking of Antioxidant properties and antimicrobial properties of *Acacia catechu* against bacterial and fungal species. Extracts of *Acacia catechu* like methanol, ethyl acetate, acetone and water were prepared by using respective chemical .In measuring the total phenolic content, Gallic acid was used as standard and its absorbance was compared with *Acacia catechu* sample. For reducing power assay of methanol extract of sample, absorbance of BHA &*Acacia catechu* was checked before & after incubation. DPPH radical scavenging activity (%) of standard and test sample was calculated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Antibacterial activity against some strains of bacteria & antifungal activity was checked.

Keywords- *Acacia catechu***,** DPPH, Total phenolic content, radical scavenging assay, BHA, Zone of inhibition.

INTRODUCTION

Acacia catechu is widely used in ayurveda for many diseases and mainly for skin diseases[1]. The chief constituents of the plant are catechin and catechutannic acid[2]. Taxifolin another important constituent has antibacterial, antifungal, antiviral, anti-inflammatory and antioxidant activity[3]. The extract of *Acacia catechu* extract have been reported to have various pharmacological effects like immune modulatory[4] antipyretic[5], hypoglycaemic, hepatoprotective activity[5,6]. It is useful in cold and cough, ulcers, boils and eruptions of the skin, bleedind piles, uterinehemorrhages, atonic dyspepsia and chronic bronchitis

etc[7,8].Seeds of *Acacia catechu* contain water soluble mucilage (6.8%); a good protein source[9].Study was conducted to evaluate the potency of *Acacia catechu* heartwood extract against dental caries causing microbes and organism associated with endodontic infections like *streptococcus mutans*, *streptoccus salivarius, Lactobacillus acidophilus* and *Enterococcus faecalis* using disc diffusion method[10].

MATERIALS AND METHODS

Plant material: Processed *Acacia catechu* sample was obtained from Bathinda district, Punjab (India). This processed *Acacia catechu* sample was used for preparation of different sample extracts to check its antioxidant and antimicrobial activity.

Preparation of plant extract: Powered form processed *Acacia catechu* sample (25 grams) was dissolved in 50 ml each of the four different solvents i.e. methanol, ethyl acetate, acetone and water. These four different extracts of processed *Acacia catechu* sample was kept at shaker at 200 rpm for 72 hours and then kept for centrifugation for 10 minutes at 5000 rpm at 10° C. It was then passed through simple filter paper. This filtrate was used for further experiments of antimicrobial activity. 20 mg *Acacia catechu* powder was dissolved in 200 ml of 50% methanol and this is used for DPPH assay to check antioxidant properties.

Preparation of standard solution: Gallic acid stock and sample stock was prepared .20 mg *Acacia catechu* powder was dissolved in 200 ml of 50% methanol and this is used to check total phenolic content. Sample extract which was prepared in four different solvents was used to check antimicrobial activity. Stock solution of DPPH was prepared by dissolving 0.66 mg of DPPH dissolved in 30 ml of 80% aqueous methanol. 1 gram of powdered *Acacia catechu* sample was dissolved in 10 ml of 80% aqueous methanol. BHA stock was prepared by adding 1 gram of BHA in 10 ml of 80% aqueous methanol. Total phenolic content of the *Acacia catechu* sample was determined by Folin-Ciocalteu method**.**

DPPH Assay: The hydrogen atom donating ability of the different plants extracts was determined from the decolorization of a purple colored methanol solution of DPPH following the method of Blois (1958)[11]. DPPH is stable nitrogen centered radical. The odd electrons in the DPPH free radical give a strong absorption maximum at 517 nm. In this assay, 1 ml of diluted *Acacia catechu* sample was mixed with 3 ml of DPPH (0.1mM) in methanol solution. The absorbance of reaction mixture at 517 nm was taken. The decrease in the absorbance was correlated with the scavenging action of the test compound. Gallic acid being a phenolic compound was used as a positive control. The radical scavenging activities were expressed as percentage of inhibition and calculated according to the following equation[12]. Percentage of DPPH inhibition= (Ac-As/Ac) ×100.

Where Ac=absorbance of control and As= absorbance of sample

Antimicrobial assay: Antibacterial activity of four different extracts of *Acacia catechu* was determined by using cup diffusion method on nutrient agar media plates[13].250ml NAM media was prepared and then autoclaved. One loop full bacterial culture was spread on the solid nutrient agar plates with sterile swab. Plates were incubated for 24 hours at room temperature for growth of bacterial culture on NAM plate. After the incubation four cups were made in each plate using cork borer (5mm) in the presence of flame. Then plant solvent extracts were loaded in the cups. Plates were kept in incubator at room temperature to check antimicrobial activity. Results or clear zones were checked after 48 hours.

The above mentioned procedure was repeated to check antifungal activity. Fungus culture was used for antifungal testing and was grown on PDA plates. After 5 days of growth cups were made in each plate using cork borer (5mm). Then four different plant extracts were loaded in four different cups in both plates. Both plates were kept in incubator to check clear zones. Observations were made after 3 days of incubation.

RESULTS AND DISSCUSSION

Total phenolic Content: Most of the phenolic or polyphenolic compounds in nature have antioxidative activities, example include tocopherol, flavonoid and other organic acid[14]. Gallic acid was taken as standard to check phenolic content of *Acacia catechu.*

Table-1: Absorbance of Gallic acid &*Acacia catechu* sample

Sample (In µg)	Absorbance of Standard Gallic acid	Absorbance of *Acacia catechu* sample
0	0	0
10	0.516	.011
15	0.809	.019
20	1.052	.007
25	1.309	.028
30	1.485	.029

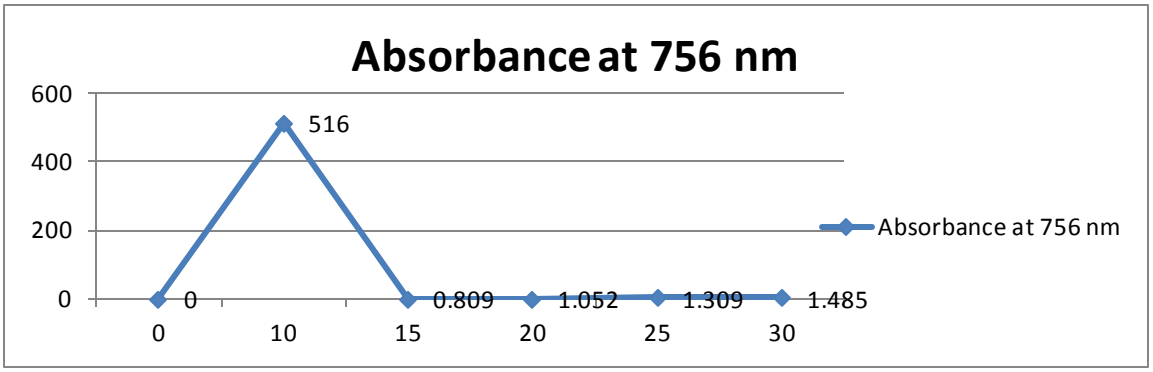

Figure-1: Graph shows absorbance of *Acacia catechu* sample at 756 nm.

Reducing power assay: The reducing activity of methanol extract of *Acacia catechu* powder was determined according to the Oyaizu method[15]. BHA was taken as standard to check the reducing power of sample. Reducing power or absorbance of sample (517 nm) was compared with standard BHA absorption, both was taken at same concentration (0.1mM).

Table-2: Absorption of BHA &*Acacia catechu* sample before incubation

Absorption of BHA(Std.) before incubation	Absorption of *Acacia catechu* before incubation
0.436	0.417

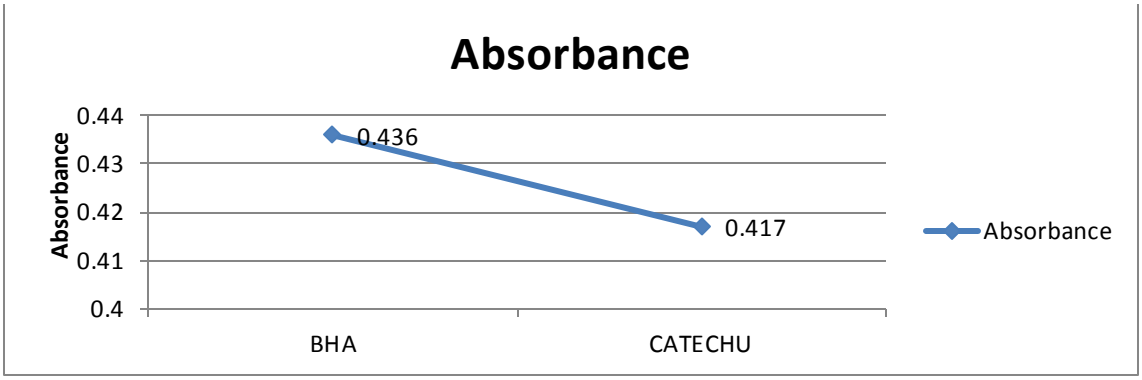

Figure-2: Graph shows absorption of BHA and *Acacia catechu* immediately (before incubation).

Table-3: Absorption of BHA &*Acacia catechu* after incubation

Absorption of BHA(Std.) after incubation	**Absorption of *Acacia catechu* after incubation (30 minutes)**
0.376	0.368

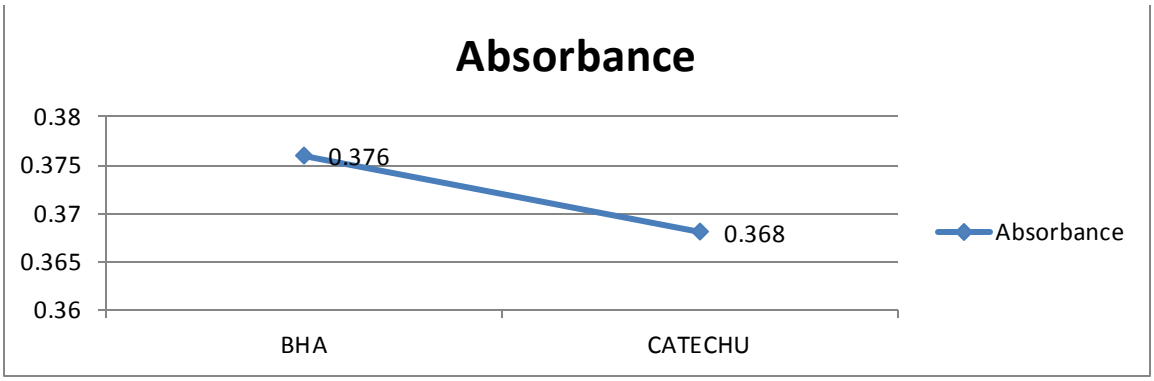

Figure-3: Graph shows absorbance of BHA and *Acacia catechu* after incubation.

DPPH radical scavenging activity: The DPPH radical scavenging assay is an easy and sensitive method for the antioxidant screening of catechu. A number of methods are available for the determination of free radical scavenging activity but the assay employing the stable DPPH free radical has maximum We have taken absorbance of standard (BHA) and sample two times, immediately and after incubation of 30 minutes.

Table-4:% DPPH radical scavenging activity before & after incubation

% DPPH radical scavenging activity before incubation (BHA+ sample)	**% DPPH radical scavenging activity after incubation (BHA+ sample)**
4.357	2.127

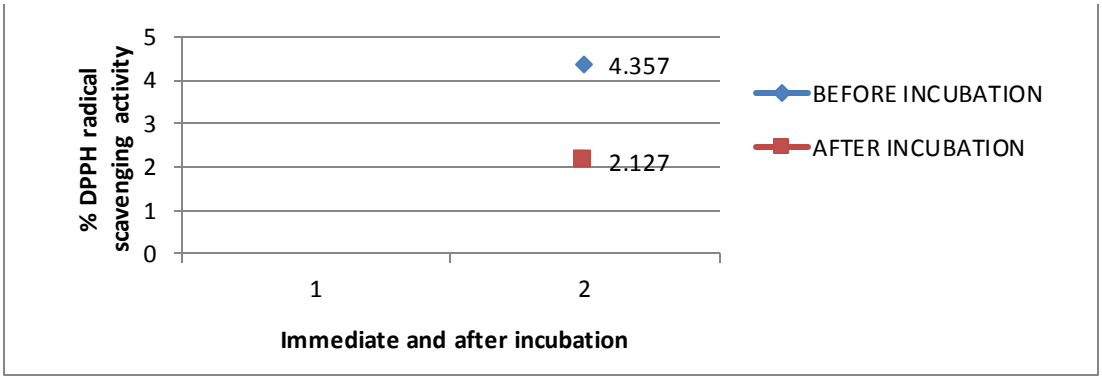

Figure-4: Graph shows % DPPH radical scavenging activity before and after incubation.

Table-5: % inhibition of BHA and sample

% inhibition of BHA	% inhibition of sample
13.76%	11.75%

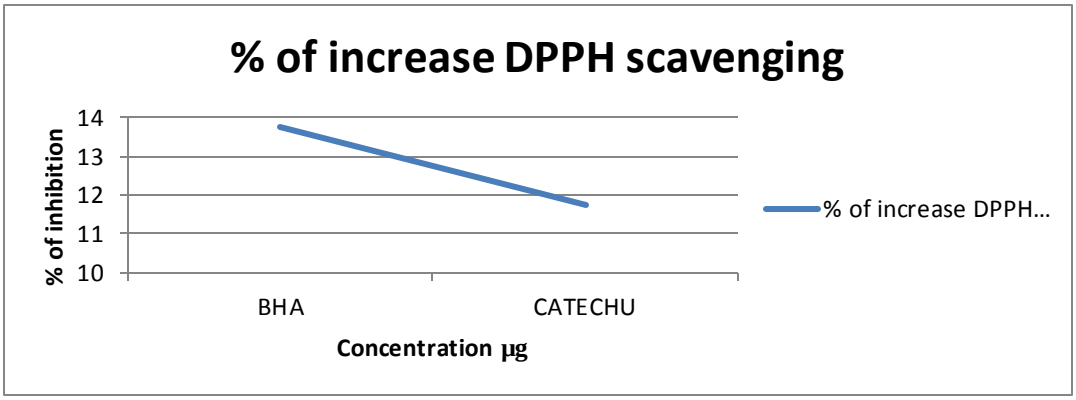

Figure-5:% of increase in DPPH scavenging activity

Antibacterial activity: Zone of inhibition of different *Acacia catechu* extracts (H_2O, Acetone, Ethyl acetate, Methanol) was measured against three bacterial strains. Sample acetone extract shows maximum zone of inhibition (1.76 cm), H_2O and methanol extracts showed same zone of inhibition (1.6 cm) and ethyl acetate extract shows minimum zone (1.1 cm) against bacterial strain 9542. Sample H_2O extract showed maximum zone (1.66 cm) against bacterial strain 3160 and ethyl acetate extract showed minimum zone of inhibition (1 cm). Acetone and methanol extracts showed 1.2 cm and 1.4 cm zones respectively against 3160 bacterial strain. Against 10636 bacterial strains, acetone extract showed maximum zone (1.6 cm) and H_2O minimum (0.6 cm), ethyl acetate and methanol extracts showed 1.16 cm and 1.266 cm respectively.

Table-6: Zone of inhibition of different *Acacia catechu* extracts against bacterial strains

Bacterial strain	Solvent extract (0.5mM)	Zone of inhibition (in cm diameter)			Mean
		B_1	B_2	B_3	
9542	H_2O	1.6	1.6	1.6	1.6
	Acetone	1.8	1.7	1.8	1.76
	Ethyl acetate	1.2	0.9	1.2	1.1
	Methanol	1.7	1.5	1.6	1.6
3160	H_2O	1.6	1.7	1.7	1.66
	Acetone	1.1	1.4	1.1	1.2
	Ethyl acetate	1.1	0.9	1.00	1
	Methanol	1.3	1.3	1.6	1.4
10636	H_2O	1.5	1.7	1.7	0.627
	Acetone	1.6	1.6	1.7	1.633
	Ethyl acetate	1.2	1.2	1.1	1.16
	Methanol	1.3	1.2	1.3	1.266

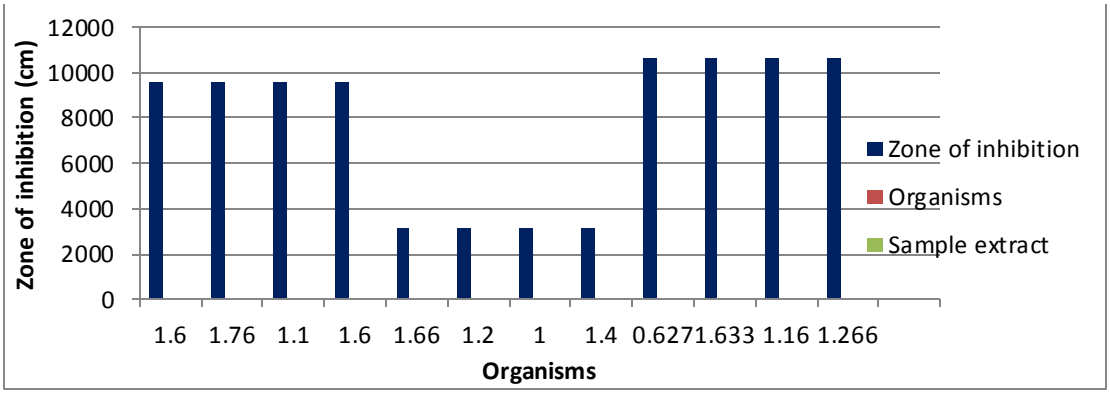

Figure-6: Graph shows Zone of inhibition of different sample extracts against different bacterial strains.

Antifungal activity: Antifungal activity was determined by measuring the zone of inhibition of different solvent extracts against fungus culture 1884. Antifungal activity of four different sample extracts against test fungus. Ethyl acetate extract showed no clear zone against test fungus.

Table-7: Zone of inhibition of different solvent extracts against fungus culture 1884.

Fungal strain	Sample extract (0.5mM)	Zone of inhibition (cm)	
		B_1	B_2
1884	H_2O	0.8	No zone
	Acetone	1.2	1.4
	Ethyl acetate	No zone	No zone
	Methanol	1.3	1.2

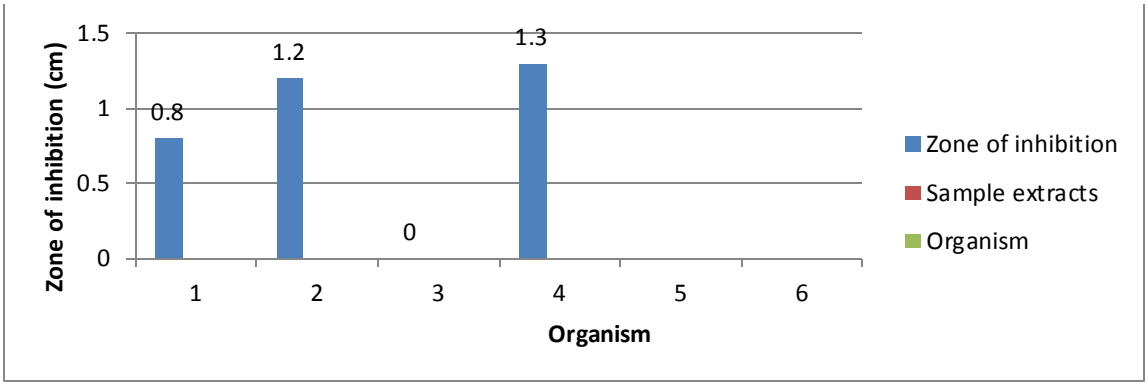

Figure-7: Graph showingzone of inhibition of different solvent extracts against fungus culture 1884.

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