World Journal of Pharmaceutical research

Volume 3, Issue 1, 525-534. Research Article ISSN 2277 – 7105

STUDIES ON PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL EFFICACY OF *ACACIA CATECHU(L.)* WILLD. AGAINST POULTRY PATHOGENS

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Article Received on
28 September 2013

Revised on 30 October 2013,
Accepted on 28 November 2013

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ABSTRACT

Acacia catechu, commonly known as catechu, cachou and black cutch is an important medicinal plant and an economically important forest tree. *A. catechu* has a great importance due to it is medicinal properties. The preliminary phytochemical analysis of leaf extracts of *A. catechu* reveals the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, sterols, saponins, tannins and terpenoids. The antimicrobial efficacy of acetone, distilled water, ethanol, ethyl acetate, methanol and n-Butyl alcohol extracts of *A. catechu* were analyzed by agar well diffusion method. The methanolic leaf extract of *A. catechu* exhibited maximum activity against *Escherichia coli*, *Salmonella pullorum*, *Trichophyton megnini* and *Aspergillus fumigatus.* Among the six extracts tested, methanol extract showed significant antimicrobial activity against the poultry pathogens.

Key words: *Acacia catechu,* leaf extracts, antimicrobial activity, poultry pathogens

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al.*, 1996). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are

collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal *et al*., 2006).

Acacia catechu Willd. (Family: Fabaceae and Sub-family: Mimosoideae) known as Black Khair and is widely distributed in different part of India. *A. catechu* grows slowly and matures to a height of about 12-15 meters. The leaves of *A. catechu* are compound. The rachis branching from the mid-rib has 4 to 5 round prickles. The rachis is nearly 10 to 20 cm long and bears 20 to 60 pinnae each about 3 to 4 cm long. The fruit of is pod shaped. It is 5 to 7 cm long and 1 to 1.5 cm wide and shining brown in color. The sap wood of is large and yellowish white and heart wood is small and red in colour (Anonymous, 2002; Wallis, 2005; Anonymous, 2004; Qadry, 2008).

The bark of *A. catechu* is strong antioxidant, astringent, anti-inflammatory, anti-bacterial and antifungal in nature. The extract of this plant is used to treat sore throats and diarrhoea, also useful in high blood pressure, dysentery, colitis, gastric problems, bronchial asthma, cough, leucorrhoea and leprosy. It is used as mouthwash for mouth, gum, sore throat, gingivitis, dental and oral infections. The heartwood is used to yield concentrated aqueous extract i.e. cutch which is astringent, cooling and digestive. It is useful in cough, ulcers, boils and eruptions of the skin. Decoction of the bark is given internally in case of leprosy. *Acacia* sp. produces gum exudates, traditionally called gum Arabic or gum Acacia, which are widely used in the food industry such as emulsifiers, adhesives, stabilizers and in chronic renal failure (Shen *et al*., 2006).

The development of an intensive poultry industry in developing countries depends primarily on the control of poultry diseases. Economic losses to poultry diseases are currently 10 to 50 percent of the gross value of production in developing countries. Hence, in the present investigation, we evaluated the phytochemical analysis and antimicrobial efficacy of *Acacia catechu* (l.) willd. against poultry pathogens.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *A. catechu* free from disease were collected from Nagapattinam District. The leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water, leaf material was then air-dried on sterile blotter under shade.

Solvent extraction

Acetone, ethanol, ethyl acetate, methanol and n-Butyl alcohol extracts of *A. catechu* were prepared according to the methodology of Indian Pharmacopoeia (Anonymous, 1966). The aqueous extraction achieved through the percolation method. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50ºC). The extracts were put in air tight containers stored in a refrigerator.

Preliminary phytochemical analysis

Preliminary phytochemical screening of the leaf extracts of *Acacia catechu* Willd. was performed as per standard procedure (Kokate *et al*., 2005; Harborne, 2005).

Screening of antimicrobial activity (Perez *et al*., 1990)

The antimicrobial activity of *Acacia catechu* was screened by agar well diffusion method.

Selection of Microorganisms

Totally ten chicken pathogenic microorganisms namely five bacterial strains such as, *Escherichia coli, Proteus vulgaris, Salmonella pullorum, Staphylococcus aureus* and *Streptococcus pyogenes* five fungal strains such as, *Aspergillus flavus, A. fumigatus, A. ochraceus, Candida albicans* and *Trichophyton megnini* were selected for the present investigation. The chicken pathogenic bacteria and fungi were originally obtained from Microbial Germ Plasm Culture Collection Unit (MGPCCU), Sri Gowri Biotech Research Academy, Thanjavur and used for the present investigation.

Antimicrobial activity

The antimicrobial activity of ethanol, acetone, ethyl acetate, methanol, n-Butyl alcohol and distilled water extracts of *Acacia catechu* were tested against the selected bacterial and fungal strains. The sterilized nutrient agar medium and potato dextrose agar medium was poured into each sterile petriplates and allowed to solidify. By using a sterile cotton swabs, a fresh bacterial and fungal culture was spread over the plates by following spread plate technique. One well of 5mm size made into the agar plates with the help of sterile cork borer, the wells were loaded with 200µl of ethanol, acetone, ethyl acetate, methanol, n-Butyl alcohol and distilled water extracts of *Acacia catechu* were loaded in to separated wells. The plates were incubated for 24 hours at 37^{o}C for bacteria and 28^{o}C for 48 - 72 hours for fungi. After the incubation period, the results were observed and the diameter of the inhibition zone was

measured around the wells. The antibiotic sensitivity test was analysed using standard antibiotics Streptomycin (10 µg/ disc) for bacteria and Fluconazole (10 µg/ disc) for fungi.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

The preliminary phytochemical analysis of ethanol, acetone, ethyl acetate, methanol, n-Butyl alcohol and distilled water extracts of *Acacia catechu* leaf were analysed. Phytochemical analysis of tested extracts revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, sterols, saponins, tannins and terpenoids except amino acids and fats (Table 1). Similarly, Deshpande (2013) reported the phytochemical analysis of *Acacia nilotica* revealed the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, cardiac glycosides and anthraquinone in ethanol and petroleum ether extracts while fixed oils and fats, proteins and amino acids were absent.

Table 1 Qualitative phytochemical analysis of leaf extract of *Acacia catechu* (L.) Willd.

S.No	Phytocompounds	Acetone	Distilled water	Ethanol	Ethyl acetate	Methanol	n-Butyl alcohol
1.	Alkaloids	+	+	+	-	+	+
2.	Amino acids	-	-	-	-	-	-
3.	Carbohydrate	-	+	+	-	-	-
4.	Fat	-	-	-	-	-	-
5.	Flavonoids	-	-	-	+	+	-
6.	Glycosides	+	+	-	-	+	-
7.	Phenols	+	-	-	-	-	-
8.	Protein	-	-	-	-	-	+
9.	Saponins	-	-	-	+	-	+
10.	Sterols	+	+	+	+	-	-
11.	Tannins	-	-	+	-	-	-
12.	Terpenoids	-	-	+	-	+	-

+ : Presence - : Absence

Antimicrobial activity

The antibacterial activity of different extracts of *Acacia catechu* L. leaf was shown in Table 2 (Plate 1&2). Acetone extract of *Acacia catechu* L. leaf exhibited minimum to moderate

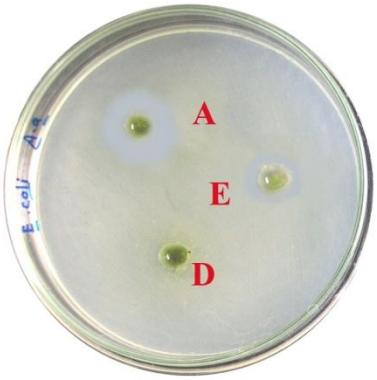
activity against the tested pathogens (Inhibition zone ranging 7 to 11 mm). Distilled water extracts showed least activity against *Salmonella pullorum* and no activity against rest of other tested pathogens. The methanol extract of *Acacia catechu* exhibited maximum inhibition zone of 12 mm against *Escherichia coli* and *Salmonella pullorum*. Methanol extract showed promising antibacterial activity when compared to other extracts. Our present results accordance with the Negi and Dave, (2011) findings. They reported methanolic extract of *Acacia catechu* L showed maximum antimicrobial activities against human pathogens.

Table 2. Antibacterial activity of *Acacia catechu* (L.) Willd.

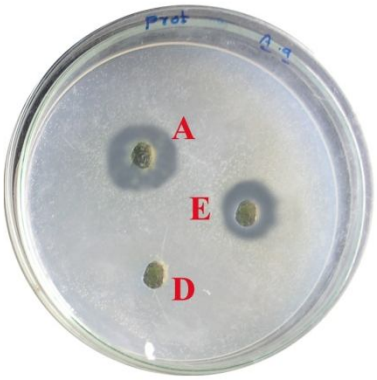
S.No	Bacterial pathogens	Zone of inhibition (diameter in mm)						
		Acetone	Distilled water	Ethanol	Ethyl acetate	Methanol	n-Butyl alcohol	Standard Antibiotic (Streptomycin sulphate)
1.	*Escherichia coli*	10.5	-	9.5	8	12	10.3	13.1
2.	*Proteus vulgaris*	8.7	-	9	-	11	10	10.5
3.	*Salmonella pullorum*	10	8.5	11	-	12	10.5	14
4.	*Staphylococcus aureus*	11	-	10	8.5	9.9	8.4	10
5.	*Streptococcus pyogenes*	8.2	-	8.6	-	7	10	19.8

The methanol leaf extract of *Acacia catechu* L. exhibited promising activity against tested fungal pathogens. The zone of inhibition range was 7 to 20 mm. The acetone (7 to 13 mm), distilled water (5-10 mm) and ethanol extracts (6 to 12 mm) showed moderate antifungal activity (Table 3; Plate 3 &4). The present investigation revealed that under the experimental conditions methanol is ideal solvent to extract antimicrobial compounds found in leaves. This finding was also supported by several workers (Eloff, 1988; Al-Bayati and AL-Mola, 2008; Negi and Dave, 2011). The overall investigation can be concluded that *Acacia catechu* L. has antimicrobial properties against the poultry pathogens.

Plate 1. Antibacterial activity of *Acacia catechu* (L.)



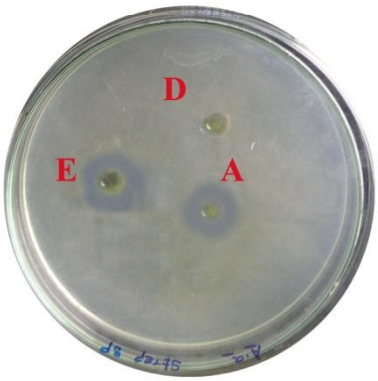
Escherichia coli



Proteus vulgaris

Salmonella pullorum

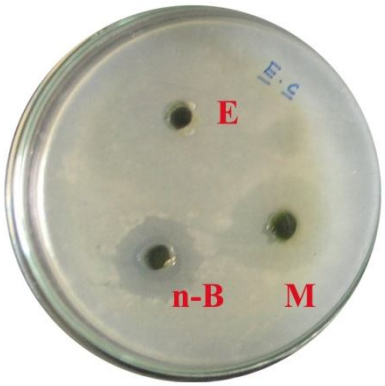
Staphylococcus aureus



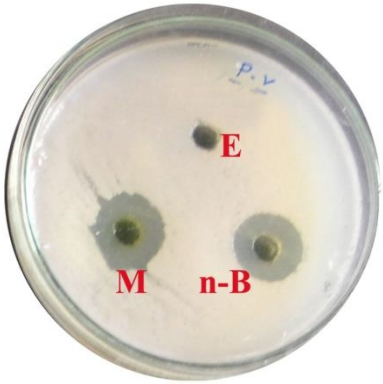
Streptococcus pyogenes

A- Acetone, D - Distilled water, E - Ethanol

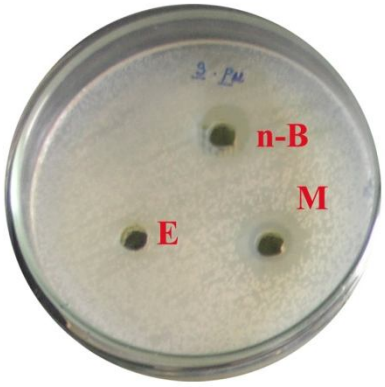
Plate 2. Antibacterial activity of *Acacia catechu* (L.)



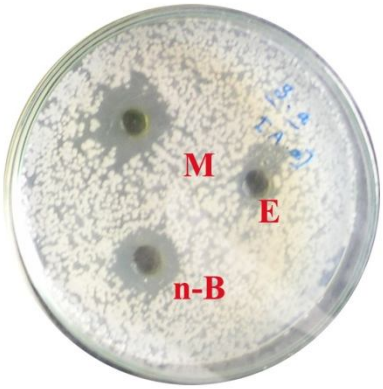
Escherichia coli



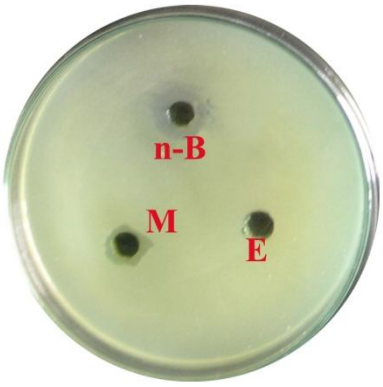
Proteus vulgaries



Salmonella pullorum



Staphylococcus aureus



Streptococcus pyogenes

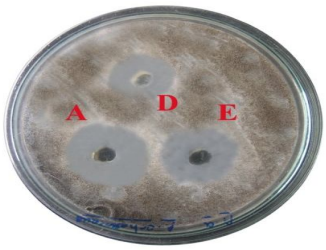
n- B - n Butyl alcohol, E - Ethyl acetate, M - Methanol

Table 3. Antifungal activity of *Acacia catechu* (L.) Willd.

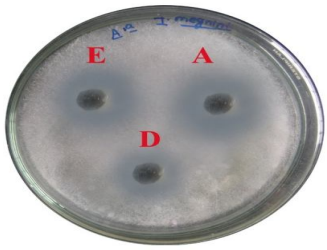
S.No	Fungal pathogens	Zone of inhibition (diameter in mm)						
		Acetone	Distilled water	Ethanol	Ethyl acetate	Methanol	n-Butyl alcohol	Standard Antibiotic Fluconazole
1.	*Aspergillus flavus*	10.3	8	9	-	10	-	10.5
2.	*A. fumigatus*	8.5	8.2	8.7	-	11.8	10	18.5
3.	*A. ochraceus*	13	10	12	-	17.5	-	14.5
4.	*Candida albicans*	10	9.4	9	-	7.5	7.3	12.3
5.	*Trichophyton megnini*	8.6	8.6	9.1	-	10	8.5	11.5

Plate 3. Antifungal activity of *Acacia catechu* (L.)

Candida albicans

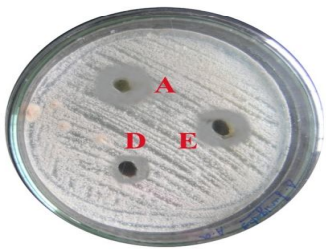


Aspergillus ochraceus



Trichophyton megnini

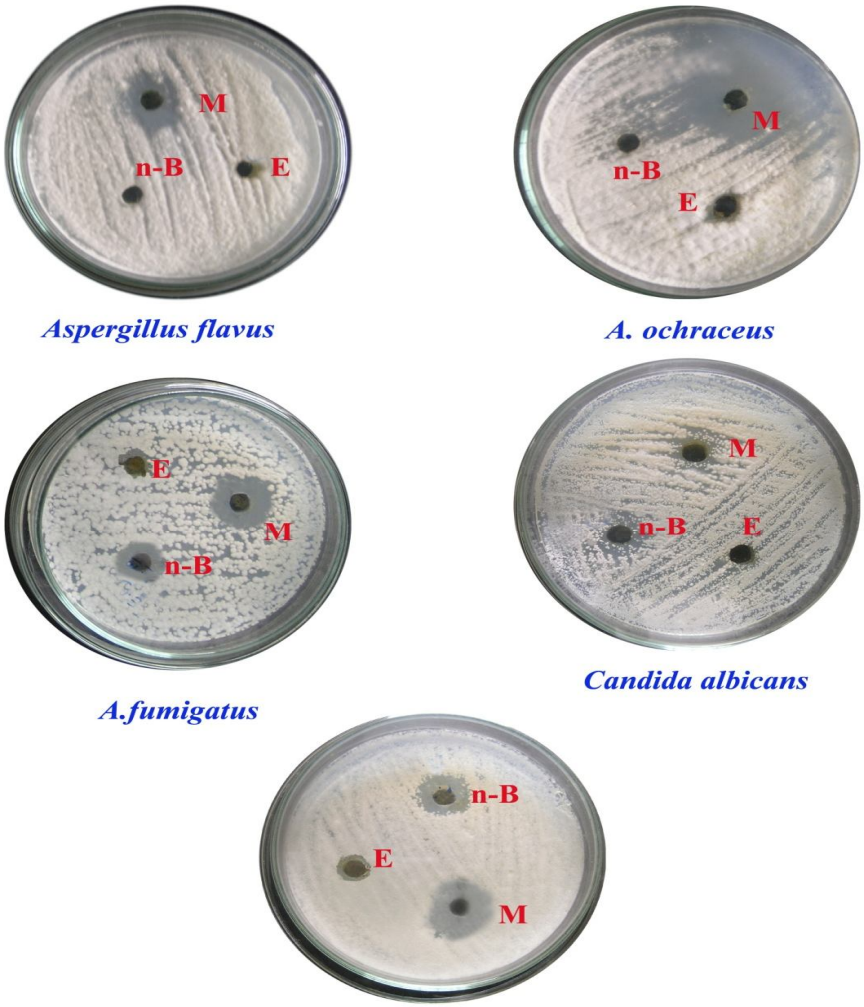
Aspergillus flavus



Aspergillus fumigatus

A - Acetone, E - Ethanol, D - Distilled water

Plate 4. Antifungal activity of *Acacia catechu* (L.)



n -B - n Butyl alcohol, E - Ethyl acetate, M - Methanol

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