Comparative Study of Antibacterial Activity of Plant Extracts of Acacia nilotica with Standard Antibiotics against Some Human Pathogenic Bacteria

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Abstract-*Plants have been used as natural sources of medicines since ancient times. Recently, due to emergence of antibiotic resistant microorganisms, demand of natural or herbal alternatives of synthetic antibiotics has increased leading to research into medicinal plants and their potential herbal antibiotics. The present study was intended to screening the plant Acacia nilotica (Family – Mimosaceae) for the significant antibacterial activities against some human pathogenic bacteria and its comparative study with standard antibiotics which was determined and evaluated by Antibiotic Susceptibility Testing using the Kirby-Bauer Disk Diffusion Method.*

Keywords- Acacia nilotica, Comparative Study, Antibacterial Activity, Herbal Antibiotics, Antibiotic Susceptibility Testing.

I. INTRODUCTION

Microbial infections are major public health problems in the developing countries. Antibiotics are used to treat these infections. Due to indiscriminate use of commercial antibiotics, the incidence of multiple antibiotic resistance in human pathogens is increasing. This has forced the scientists to search for new antimicrobial substances from various sources like medicinal plants. Medicinal plants constitute the main source of new pharmaceuticals and health care products. The use of traditional medicines is widespread in India. Acacia nilotica (Family-Mimosaceae) is a multipurpose plant. It is used for treatment of various diseases (Singh et al., 2009b). It serves as the source of polyphenols. The plant contains a profile of a variety of bioactive components (Singh et al., 2009a). The bark of plant is used extensively for colds, bronchitis, diarrhoea, bleeding piles and leucoderma (Del, 2009). Pods and tender leaves are given to treat diarrhoea and are also considered in folk medicine to treat diabetes mellitus (Gilani et al., 1999).

Acacia nilotica grows to 15-18 m in height and 2-3 m in diameter. In India, stem circumference can reach 2-3 m, with a clear bole height of 6-7.5 m (Troup and Joshi, 1983). Acacia nilotica is widespread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India.

II. RESEARCH METHODOLOGY

1. Plant Material/ Bacterial Isolate Source:

The different plant parts of Acacia nilotica i.e. Leaves, Stem Bark and Fruit (Seed Pods) were obtained from fields of Kota Area, Bilaspur and 5 different pathogenic bacterial isolates were obtained from Lab One Metropolis, Raipur.

2. Extraction of Plant Material:

The collected plant parts i.e. leaves, stem bark and fruit of Acacia nilotica were washed with running tap water and then, left for being air dried at room temperature.

Extraction of the Plant Material was carried out by Simple Maceration Process. The extracts of each plant part i.e. leaves, stem bark and fruit of Acacia nilotica were extracted using Methanol (70%), Distilled (Autoclaved) Water and Chloroform. The dried plant parts were crushed in mortar-pestle thoroughly and then, 10 grams of the crushed leaves, stem bark and fruit were each dissolved in 100 ml of the mentioned solvents and were left for 72 hours at normal room temperature. The suspensions were filtered using whatman filter paper no.1 and the filtrates (extracts) were delivered into sterile, clean containers with suitable labelling and kept at 4°C until use.

Thus, 3 different types of plant extracts i.e. methanolic, aqueous and chloroform extracts (100 mg/ml) were obtained from each plant part.

3. Bacterial Species Used for Antibiotic Susceptibility Testing:

Five species of bacteria were used in the study which was collected from Lab One Metropolis, Raipur, Chhattisgarh, India. These bacterial species were as Follows:

i) Staphylococcus aureus (Gram Positive)

ii) Proteus vulgaris (Gram Negative)

iii) Escherichia coli (Gram Negative)

iv) Pseudomonas aeruginosa (Gram Negative)

v) Klebsiella pneumonia (Gram Negative)

All the above bacterial species were maintained on nutrient agar slants at 4°C.

4. Preparation of Inoculum:

Nutrient Broth medium was prepared by dissolving 0.4g/50ml of distilled water for the growth of bacterial inoculum. Subsequently, the pH was adjusted at 7.0 and then, the broth was autoclaved at 121° C for 15 minutes. The selected human pathogenic bacteria were activated by culturing in Nutrient Broth by incubating some colonies in the broth for 24 hours at 37° C.

5. Preparation of Muller-Hinton Agar (MHA) Plates:

Muller-Hinton Agar (MHA) was used as a base medium for Antibiotic Susceptibility Testing using the Kirby-Bauer Disk Diffusion Method. All the ingredients of MHA in the appropriate quantities were suspended in one litre of distilled water (Table 1.)

Table 1. Composition of Muller-Hinton Agar (MHA)

Ingredients	In Gram/Litre
Beef Extract	2.00 gm.
Acid Hydrolysate of Casein	17.50 gm.
Starch	1.50 gm.
Agar	17.00 gm.
Distilled Water	1000 ml

After adjusting the pH at 7.3 ± 0.1 at 25ºC, the medium was heated with frequent agitation and boiled for one minute to completely dissolve the medium. Then the medium was Autoclaved at 121°C for 15 minutes and subsequently, cooled to room temperature. The cooled MHA was then poured into sterile petri dishes on a level, horizontal surface to give uniform depth. Then allowed the plates to cool to room temperature and stored the plates at 2-8 ºC until use.

6. Inoculation of the Bacterial Species on MHA Plates:

The turbidity of the inoculums of the 5 bacterial species was compared with that of 0.5 McFarland Turbidity Standard to estimate the bacterial density.

0.5 McFarland Turbidity Standard was prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulphuric acid. The bacterial inoculums were further diluted with normal saline solution or incubated further as necessary to attain comparative turbidity. After attainment of comparative turbidity with 0.5 McFarland Turbidity Standard, the inoculums of the 5 bacterial species will have 106 - 108 CFU ml-1.

After attainment of comparative turbidity with 0.5 McFarland Turbidity Standard, the inoculums of the 5 bacterial species were evenly spread by the use of sterile cotton-swabs by streaking method. Each bacterial species was inoculated in 10 plates of MHA. Thus, a total of 50 plates of MHA were inoculated with the 5 bacterial species.

7. Preparation of Antibiotic Discs and Negative Controls:

Discs of 6 mm in diameter were cut from whatman filter paper no. 1 using a paper borer. After that, the prepared discs were put in suitable containers and then, the discs were subjected to autoclave in order to sterilize the discs (adjusting the conditions of autoclave to be 121°C for 15 minutes) and left to become cold.

The 3 different plant extracts (of each plant part) previously prepared were taken and the discs were placed in these extracts (for about 1 minute) using sterilized forceps so that each disc could absorb adequate quantity. Thus, 3 different types of antibiotic discs were prepared from the 3 different plant extracts (of each plant part) and the produced discs (each one) have the ability to absorb about 0.01ml.

Prepared discs were stored at 4°C in the refrigerator till use. For negative control methanol paper discs were prepared by dipping the disks into methanol which were used for the extraction processes.

The standard antibiotic disks which were later used for comparing with the antibacterial activities of the plant extracts contained Ceftriaxone (30 mcg), Tetracycline (30 mcg), Cefuroxime (30 mcg), Ciprofloxacin (5 mcg), Rifampicin (5 mcg), Gentamicin (10 mcg), Ampicillin (10 mcg), Levofloxacin (5 mcg) in each disc.

8. Antimicrobial Susceptibility Testing by Kirby-Bauer Method:

The antibiotics susceptibility procedure for bacterial species has been done through using a method that depends on the ability of disc to permit the penetration of antibiotics through the medium which is also called Kirby-Bauer Method (Kirby-Bauer, 1996).

The overall steps of the procedure were produced through entirely sterilized status. In this method, the prepared 3 different types of antibiotic discs obtained from methanolic, aqueous and chloroform extracts of each plant part, were fixed under sterilized conditions on each MHA plate inoculated with each bacterial species. Thus, a total of 15 MHA plates were prepared for the 5 different bacterial species (3 x 5 = 15). The methanol paper discs were used as negative controls on the plates.

The organisms under the experiment were evaluated, as well, for their susceptibility toward some standard antibiotics including: Ceftriaxone (30 mcg), Tetracycline (30 mcg), Cefuroxime (30 mcg), Ciprofloxacin (5 mcg), Rifampicin (5 mcg), Gentamicin (10 mcg), Ampicillin (10 mcg), Levofloxacin (5 mcg) again by disc diffusion procedure.

For this test, the standard antibiotic discs of the above mentioned antibiotics were fixed under sterilized conditions on 5 MHA plates inoculated with each of the 5 different bacterial species.

9. Incubation and Assessment of the Antibacterial Activity:

After the prepared 3 types of antibiotic discs were applied on each MHA plate, the plates were then incubated upside down at 37°C for 24 hours. After 24 hours of incubation, the diameter of the clear zones of inhibitions was measured by a ruler. The inhibition zones were measured and recorded. The assessment of antibacterial activity was based on the measurement of the diameter of the inhibition zone formed around the disc. All the procedure mentioned above was carried out in two replicates and the Mean Diameter of Inhibition Zone (MDIZ) was calculated and tabulated. The MDIZ formed were compared to the diameters of inhibition zones obtained by standard antibiotics* against the 5 different bacterial species. The results of antimicrobial susceptibility tests were interpreted as Sensitive (> 18 mm), Moderately Sensitive (14-18 mm) and Resistant (< 14 mm).

*The standard antibiotics were in the form of discs containing micrograms (mcg) of the antibiotics as per CLSI and EUCAST.

III. RESULTS

A. Assessment of the Antibacterial Activity of A. nilotica Leaf Extract:

MDIZ (In Millimetres)				
S.No.	**Bacterial Species**	**Solvent Used for Extraction of Leaf Extract**		
		Methanol (70%)	Distilled Water	Chloroform
1.	*Staphylococcus aureus*	27	18	14
2.	*Proteus vulgaris*	25	20	18
3.	*Escherichia coli*	24	20	17
4.	*Pseudomonas aeruginosa*	31	24	20
5.	*Klebsiella pneumonia*	33	21	16

Table 2. Mean Diameter of Inhibition Zone (MDIZ) Obtained by Using A. nilotica Leaf Extract

B. Assessment of the Antibacterial Activity of A. nilotica Bark Extract:

MDIZ (In Millimetres)				
S.No.	**Bacterial Species**	**Solvent Used for Extraction of Stem Bark Extract**		
		Methanol (70%)	Distilled Water	Chloroform
1.	*Staphylococcus aureus*	29	21	18
2.	*Proteus vulgaris*	25	20	20
3.	*Escherichia coli*	27	23	14
4.	*Pseudomonas aeruginosa*	33	25	19
5.	*Klebsiella pneumonia*	28	24	17

Table 3. Mean Diameter of Inhibition Zone (MDIZ) Obtained by Using A. nilotica Stem Bark Extract

C. Assessment of the Antibacterial Activity of A. nilotica Fruit Extract:

MDIZ (In Millimetres)				
S.No.	**Bacterial Species**	**Solvent Used for Extraction of FruitExtract**		
		Methanol (70%)	Distilled Water	Chloroform
1.	*Staphylococcus aureus*	21	20	12
2.	*Proteus vulgaris*	20	20	18
3.	*Escherichia coli*	18	15	15
4.	*Pseudomonas aeruginosa*	23	17	13
5.	*Klebsiella pneumonia*	25	18	14

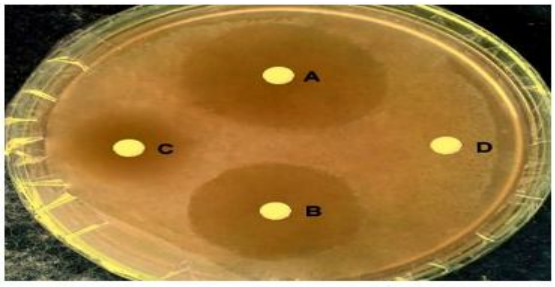
Table 4. Mean Diameter of Inhibition Zone (MDIZ) Obtained by Using A. nilotica Fruit Extract

D. Assessment of the Antibacterial Activity of Standard Antibiotics:

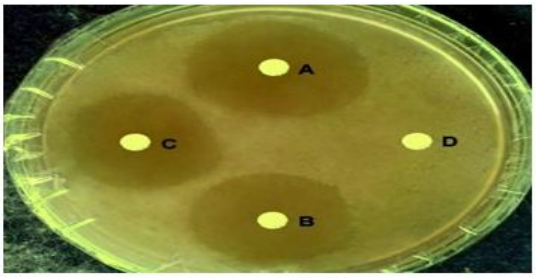
Diameter of Inhibition Zone (In Millimetres)						
S.No.	**Standard Antibiotics**	**Bacterial Species**				
		S. aureus	*P. vulgaris*	*E. coli*	*P. aeruginosa*	*K. pneumonia*
1.	CTR (30 mcg)	18	22	24	20	23
2.	TE (30 mcg)	26	15	22	0	17
3.	CXM (30 mcg)	29	20	21	0	22
4.	CIP (5 mcg)	23	32	35	31	29
5.	RIF (5 mcg)	33	5	8	0	7
6.	GEN (10 mcg)	22	24	25	21	20
7.	AMP (10mcg)	29	21	17	0	23
8.	LE (5 mcg)	28	33	36	24	37

Table 5. Diameter of Inhibition Zone Obtained by Using Standard Antibiotics

Abbreviation	Name of the Antibiotic
CTR	Ceftriaxone(30 mcg)
TE	Tetracycline (30 mcg),
CXM	Cefuroxime (30 mcg),
CIP	Ciprofloxacin (5 mcg),
RIF	Rifampicin (5 mcg),
GEN	Gentamicin (10 mcg),
AMP	Ampicillin (10 mcg),
LE	Levofloxacin(5 mcg),



Photograph 1.



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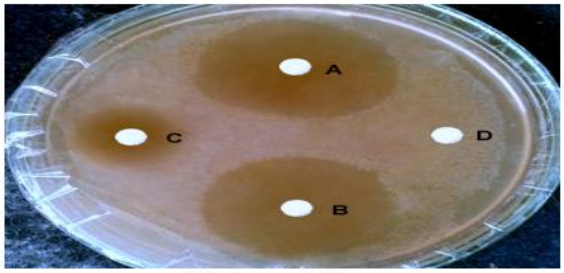

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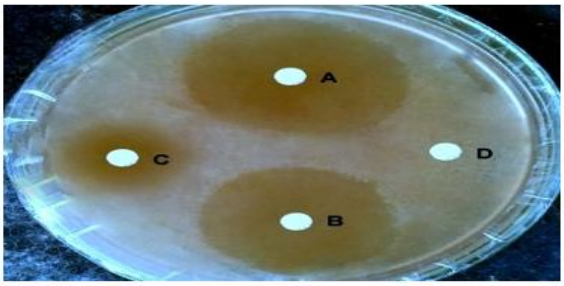

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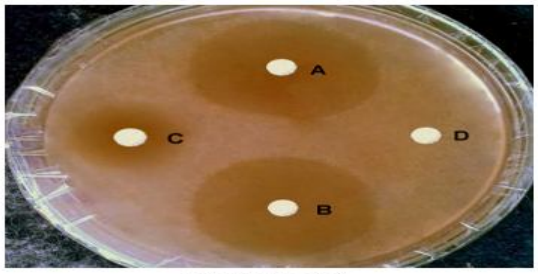
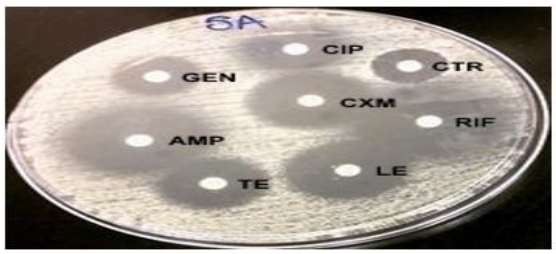


Photograph 5.

Photographs (1 – 5): Plates Inoculated with S. aureus (1), P. vulgaris (2), E. coli (3), P. aeruginosa (4) and K. pneumonia (5) showing different Zones of Inhibition against Methanolic (A), Aqueous (B) and Chloroform (C) Extracts of A. nilotica Stem Bark. (D) is the Control Disc containing Methanol.



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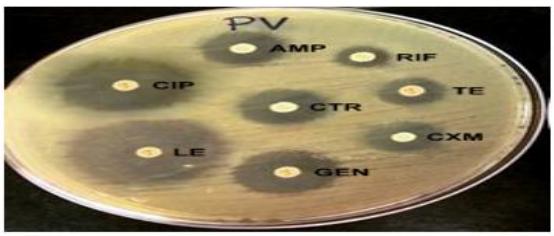

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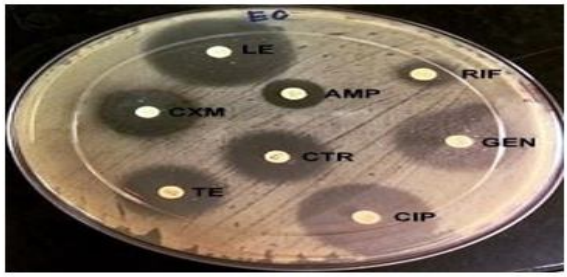

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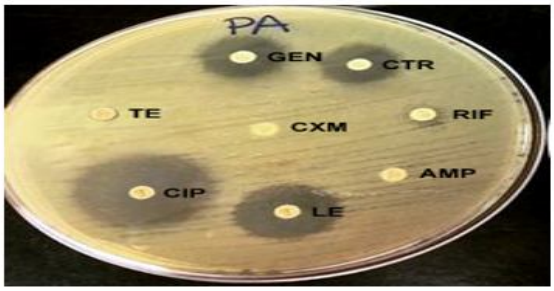

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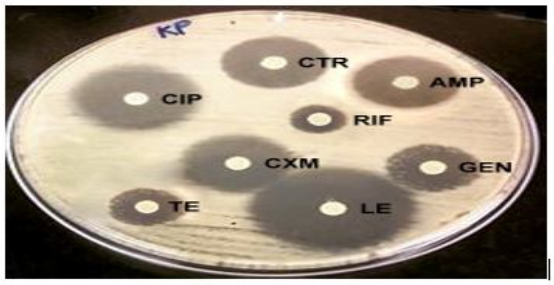

Photograph 10.

Photograph (6 – 10): Plates Inoculated with S. aureus (6), P. vulgaris (7), E. coli (8), P. aeruginosa (9) and K. pneumonia (10) showing different Zones of Inhibition against Ceftriaxone (CTR), Tetracycline (TE), Cefuroxime (CXM), Ciprofloxacin (CIF), Rifampicin (RIF), Gentamicin (GEN), Ampicillin (AMP), Levofloxacin (LE).

IV. DISCUSSIONS

The antimicrobial activity of Acacia nilotica has been well established by many researchers in different parts of the world. However, the activity of A. nilotica ssp. in the Indian State of Chhattisgarh has not been clearly elucidated.

In this study, 100 mg/ml of methanolic, aqueous and chloroform extracts of leaves, stem bark and seed pods of A. nilotica were evaluated for their antimicrobial activity against some human pathogenic bacteria. Table 2. demonstrates that the leaves methanolic extract of A. nilotica exhibited high antimicrobial effect which was about 27 mm, 25 mm, 24 mm, 31 mm and 33 mm against standard strains of Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia, respectively, while the aqueous extract of the same plant part showed less activity against the same strains which was 18 mm, 20 mm, 20 mm, 24 mm and 21 mm for the above mentioned strains, consecutively. The chloroform extract of the same plant part did not show any significant activity against the same strains which was 14 mm, 18 mm, 17 mm, 20 mm and 16 mm for the above mentioned strains, consecutively.

Similar result was obtained with the stem bark and fruit extracts as demonstrated in Table 3. and Table 4. Methanolic stem bark and fruit extracts were more effective than the aqueous extracts, and the chloroform extracts were found to be the least effective against the above mentioned strains. This may be due to the fact that methanol is strongly capable of extracting active component responsible for antibacterial activity than the distilled water and chloroform. Thus, only the methanolic extracts of A. nilotica leaves, stem bark and fruit were used for further comparative studies with standard antibiotics.

Saini M.L. (2008) examined antimicrobial activity of Acacia species and A. nilotica in comparative study, which exhibited highest activity against E. coli and S. aureus in methanolic extract. Mashram N. (2009) has observed the anti-microbial activity of Acacia nilotica, against S. aureus, B. subtilis and E. coli. The leaf and bark extracts showed zone of inhibition between 7.5-16 and 8-15.5 mm, respectively and most active against E. coli. Agrawal et al. (2010) had earlier reported that the anti-microbial activity of this plant may be due to presence of biologically active constituents such as tannin, alkaloids, flavonoids, phenolic compounds, saponins and cyanogenic glycosides which have been documented as a potent anti-microbial agent. It was also reported that the plant was found to be rich in gallic acid, methyl gallate and catechin. The highest antimicrobial activity of methanolic extract could be due to the presence of high amount of phenolics and flavonoids.

On comparing the antibacterial activity of the plant extracts to that of the standard antibiotics (Table 5.), it was observed that the methanolic extract of A. nilotica leaves was considerably effective against Staphylococcus aureus than Ceftriaxone and Gentamycin; against Proteus vulgaris than Tetracycline, Cefuroxime and Rifampicin and against Escherichia coli than Rifampicin and Ampicillin. It was considerably more effective against Pseudomonas aeruginosa and Klebsiella pneumonia than nearly all of the standard antibiotics. However, the methanolic extract of A. nilotica stem bark was considerably effective against Staphylococcus aureus than Ceftriaxone, Ciprofloxacin and Gentamycin; against Proteus vulgaris than Tetracycline, Cefuroxime and Rifampicin and against Escherichia coli than Tetracycline, Cefuroxime, Rifampicin and Ampicillin. It was considerably more effective against Pseudomonas aeruginosa than all of the standard antibiotics and against Klebsiella pneumonia than nearly all of the standard antibiotics except Levofloxacin. The methanolic extract of A. nilotica fruit was not found to be considerably effective against Staphylococcus aureus than the standard antibiotics but was effective against Proteus vulgaris than Tetracycline and Rifampicin and against Escherichia coli than Rifampicin only. It was considerably more effective against Pseudomonas aeruginosa than nearly all of the standard antibiotics except Ciprofloxacin and against Klebsiella pneumonia than nearly all of the standard antibiotics except Levofloxacin. Thus, the methanolic extract of A. nilotica stem bark was the most effective among all the extracts against the human pathogenic bacteria studied.

V. CONCLUSIONS

Acacia nilotica has been in use since ancient times to treat wide range of diseases in traditional system medicine. Experimental studies have proven its antidiabetic, antihypertensive, antispasmodic, antibacterial, antifungal activity, antiplaque, antioxidant, antispasmodic, antiviral activity, catalytic and galactagogue. The scientific studies have proven the claims of traditional system of medicine. The present study has also confirmed the potential antibacterial activity of methanolic extracts of Acacia nilotica leaves, stem bark and fruit against the selected human pathogenic bacteria which is consistent with other studies previously done. This could be due to the presence of enormous amount of flavonoids and phenolic compounds, which are responsible for the immense antimicrobial property of the methanolic extracts.

Since, A. nilotica is a commonly available plant; it may represent a potential, economical therapeutic agent for infectious diseases, due to its antimicrobial activities. This plant showed potent antibacterial activity. This would be helpful to create awareness among people for taking control measures based on herbal plants against infectious diseases. Herbal based medicines can be recommended alternate to antibiotics. But, further detailed clinical researches are needed to explore its medicinal value in order to establish it as a standard drug. Moreover, as Pseudomonas aeruginosa is resistant to most of the synthetic antibiotics, the potential effectiveness of methanolic extracts A. nilotica against Pseudomonas aeruginosa can be exploited for the development of novel antibiotics against it and other susceptible species if further research, purification and concentration of the herbal extracts of Acacia nilotica are initiated. Also, this study may be the first preliminary report on the antimicrobial activity of Acacia nilotica in the state of Chhattisgarh.

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