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BIOACTIVITY GUIDED FRACTIONATION AND PHYTOCHEMICAL ANALYSIS OF *ACACIA NILOTICA*

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ABSTRACT

The traditional medicine involves the use of different plants and their bioactive constituents to treat disease and infection. *Acacia nilotica* is a multipurpose plant belonging to the family *Fabaceae*. It has been used traditionally to treat infections. The current study aimed at investigating presence of phytochemical constituents and antimicrobial activity of *Acacia nilotica* leaves extract (Methanol, Chloroform and Water) against multi drug resistant *Escherichia coli, Pseudomonas, Enterobacter, Staphylococcus aureus and Candida albican*. Extract of *Acacia nilotica* were prepared using Methanol, Chloroform and Water were screened for the antimicrobial activity using agar well diffusion method. The crude extract was further subjected to phytochemical analyses and Thin layer chromatography (TLC). Methanolic extract of leaves of *Acacia nilotica* were more active against all five pathogens. It gives highest 24mm zone of inhibition (ZOI) against Pseudomonas. Chloroform extract of leaves of *Acacia nilotica* only active against *Staphylococcus aureus.* It gives 13mm zone of inhibition (ZOI) against *Staphylococcus aureus.* Water extract of leaves of *Acacia nilotica* only active against *Staphylococcus aureus*. Water extract gives highest 16mm zone of inhibition (ZOI) against *Staphylococcus aureus*, whereas Methanolic and Chloroform extract gives zone of inhibition (ZOI) 15mm and 13mm. The Methanol, Chloroform and Water extract of leaves were used for the phytochemical analysis to find out the phytochemical constituents in the *Acacia nilotica*. The results of the phytochemical analysis of the *Acacia*

nilotica showed that alkaloids, glycoside, seponins, phytosterol, phenol, tannin, flavonoid, protein and Quinone's present in leaves extract.

Keywords: Phytochemical analysis, *Acacia nilotica*, Medicinal plants, Antimicrobial activity, *Escherichia coli, Pseudomonas, Enterobacter, Staphylococcus aureus and Candida albican*

INTRODUCTION

India is blessed with a rich abundance of medicinal plants. These plants have made a decent contribution to the advancement of ancient Indian material therapeutic. Indian subcontinent is widely considered as huge repository of indigenous medicinal plant, these plants derived products have wide application in therapeutics, treatment and utilized as traditional medicine **[1]**. Primary health care system has shifted to the use of traditional medicine **[2]**. Medicinal plants used in the treatment of diseases are as old as civilization **[3]**. Acceptance and demand of medicinal plants have increased without them survival of human and other organisms will be difficult. Herbs are health indicator of ecosystem **[4]**. According to World Health Organization (2011), medicinal plants would be the best source to obtain variety of drugs. About 80% of populations from developed countries use traditional medicines, which have compounds derived from medicinal plants **[5]**. Natural sources, such as plants and their products are rich source of secondary metabolites such as alkaloids, phenolic acids, tannins, flavonoids and other small compounds, plants can be of interest in therapeutics. Phytochemicals and various plant extracts have potential for the development of new drugs effective against infections and multidrug-resistant organisms **[6]**.

Acacia nilotica belonging to the subfamily *Mimosoideae* of the family *Fabaceae*, described by the Swedish botanist *Carl Linnaeus* in 1773. *Acacia nilotica* also known as Indian gum Arabic, Black babool- English, Acacia, Acacia Arabica- Latin, Babhul - Hindi and Napalese, Kikkar- Punjabi, Sac- Kashmiri, Babool - Unani, Babool Baum - German, Babhoola - Sanskrit, Babul, Babul Tree, Huanlong Kyain - Burmese, Kikar, Indogom - Japenese and Ummughiion – Persian **[7, 8]**. *Acacia nilotica*, Scented Thorn Acacia, is local from Egypt, over the Maghreb and Sahel, south to Mozambique and Natal, and east through Arabian Peninsula to Pakistan, India and Burma. It has gotten broadly naturalized outside its local range including Zanzibar, and Australia **[9]**.

Acacia nilotica can provide nutrients and therapeutic ingredients to treat many disease or conditions. *Acacia nilotica* leaves are used to treat wounds, phyaryngitis, bronchitis and diabetes also treat diarrhoea, Aphrodisiac, dressing of

ulcers, anti-inflammatory and Alzheimer's diseases **[10-14]**. Bark is used for bronchitis, diarrhoea, cold, antioxidant, anti-mutagenic, Anti- bacterial, astringent, acrid cooling, cytotoxic, styptic, emollient, anthelmintic, aphrodisiac, expectorant, emetic, nutritive, in haemorrhage, wound ulcers, leprosy, diuretic, small pox, skin diseases **[10, 15-19]**. *A.nilotica* gum is used as Astringent, emollient, liver tonic, antipyretic and anti-asthmatic **[20]**. Pod has Anti-diarrhoeal, Anti-hypertensive and antispasmodic, anti-fertility, astringent and against HIV-1 PR, Inhibited HIV-1 induced cytopathogenicity, antiplatelet aggregatory activity and antioxidant activity **[21, 22]**. Phytochemical screening of *Acacia nilotica* exposed that thr plant contains Flavonoids, hydrolysable and condensed tannins, amines and alkaloids, cyclitols, cyanogenic glycosides, fatty acids and seed oils, gums, fluoroacetate, non-protein amino acids, terpenes (including essential oils, diterpenes, phytosterol and triterpenegenins and saponins **[23]**.

MATERIALS AND METHODS

Sample collection

The leaves of *Acacia nilotica* was collected from Latipura village, Padra Taluka, Vadodara District- Gujarat. Fresh and Mature leaves were used in the study of phytochemical analysis and antimicrobial activity.

Test Organisms

The multi-drug resistant pathogens (*Escherichia coli, Pseudomonas, Enterobacter, Staphylococcus aureus* and *Candida albican*) were obtained from Parul Sevashram Hospital, vaghodia.

Preparation of *Acacia nilotica* leaves extracts [24]

Leaves of *Acacia nilotica* were rinsed with distilled water and kept under shade in open air for 48hours. Extraction from dried leaves was carried out by simple maceration method. The leaves were taken and grounded to coarse powder. Extract was prepared in three different solvents water, methanol and chloroform.

100g dry leaves powder was suspended in 200ml of 80%Methanol, Chloroform and Water kept for 3 to 4 days at 60°C in extraction bottle. After 3 to 4 days, mixture was filtered twice, using muslin cloth. Methanol, Water and Chloroform was then completely evaporated by natural means to obtain extract. Extract were stored in a refrigerator at 4°C respectively.

Media preparation

The media used in this study were Mueller Hinton agar (MHA). The media were prepared according to manufacturer's instructions using standard aseptic technique.

Preparation of standard antibiotic

Ampicillin 500g was prepared immediately beforehand by diluting in 1ml dimethyl sulphoxide (DMSO).

Determination of antimicrobial activity [25]

During this study antimicrobial activity of *Acacia nilotica* leaves extract were carried out by Agar well diffusion method. Mueller Hinton agar (MHA) were prepared in which 24hours old broth culture of selected organisms were added. The MHA poured into sterile petri plates and allowed to solidify. Consequently, using sterile borer, well was made into each Mueller Hinton agar plate 5 wells were made and fifty microliter of different leaves extract (methanol, chloroform and water) and same volume of control negative control(DMSO) and positive control (Ampicillin) was filled in the wells with the help of micropipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. After incubation the diameters of the results and growth inhibition zones where measured.

Phytochemical analysis

All the extract of leaves of *Acacia nilotica* was subjected to qualitative test for the identification of various plant constituents.

Detection of alkaloids by Hager's test [26]

Plant extract were treated with Hager's reagents (saturated picric acid solution). Formation of yellow coloured confirmed the Presence of alkaloids.

Detection of carbohydrate by Benedict's test [26]

Plant extract were treated with Benedict's reagent and heated gently. Presence of reducing sugars confirmed by formation of orange red precipitate.

Detection of Glycoside

A**) Legal's Test [26]**

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides. Plant extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. The presence of cardiac glycosides confirmed by Formation of pink to blood red colour.

B) Glycosides [27]

5ml of diluted sulphuric acid was added in extracts in a test tube and boiled for fifteen minutes in a water bath. It was then cooled and neutralized with 20% potassium hydroxides solution. A mixture of 10ml of equal parts of Fehling s solution A and B were added and boiled for five minutes. The presence of glycosides confirmed by formation of more dense red precipitate.

Detection of saponins by Foam Test [26]

0.5 g of plant extract was shaken with 2 ml of water. If foam produced and holds for ten minutes it indicates the presence of saponins**.**

Detection of Phyto-Sterols by Salkowski test [28]

2 ml extract taken in a test tube and add 2ml chloroform and 2ml conc. Sulphuric acid in it. Brown or red colour ring on the sulphuric acid layer given the confirmatory test for phytosterols.

Detection of phenols by Ferric Chloride Test [26]

Plant extracts were treated with 3-4 drops of ferric chloride solution. The presence of phenols confirmed by Formation of bluish black colour.

Detection of Tannins by Braymer's test [29]

2 ml of plant extract was allowed to react with 10% alcoholic ferric chloride solution. Formation of blue or greenish colour of the solution indicates the presence of the tannins.

Detection of Flavonoids [27]

5 ml of the ammonia solution was added to the portion of the plant extract. The appearance of the yellow fluorescence examined under the UV light indicated the presence of flavonoids.

Detection of Amino acid by Ninhydrin Test [26]

0.25% w/v Ninhydrin reagent was added into the plant extract and boiled for few minutes. The presence of amino acid confirmed by Formation of blue colour.

Detection of Protein by Xanthoproteic Test [26]

2ml plant extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour confirmed the presence of proteins.

Detection of Coumarins [29]

2 ml of plant extract were treated with 3 ml of 10% NaOH. Observed the formation of yellow colour indicating the presence of coumarins.

Detection of Quinones [29]

1 ml of extract was added to the 2 ml of dilute NaOH. Formation of blue green or red coloration confirms the presence of Quinones.

TLC (Thin layer chromatography)

The Silica Gel G used as an adsorbent for preparation of thin layer plate as a stationary phase.30 g powder of Silica Gel G was mixed with 80ml Distilled water. This Silica Gel G suspension was spread with a spreader on thin layer chromatographic glass plates fixed on a stage. The prepared plates were air-dried and activated in an oven at 110°C for 30 min. The activated plates then used for the application of samples. Aliquots of each of the extracts (Methanol, Chloroform and Water) were separately applied to the plate as a 6 mm wide band, 8 mm from the bottom. The 200ml mobile phase consisted of Methanol and Water (1:1). Development was carried out in a twin glass chamber saturated with the mobile phase and after which the plate was air-dried for 10 min and viewed under normal light.

The Rf values of Standard spot and Sample spots were calculated by the formula:

Rf Value = Distance from the starting point to the centre of the spot on the TLC plate / Distance from the starting point to the solvent front

RESULTS AND DISCUSSION

Table 1 shows the results of antimicrobial activity of leaves extract (Methanol, Chloroform and Water) of *Acacia nilotica* against *Escherichia coli, Pseudomonas, Enterobacter, Staphylococcus aureus, Candida albicans.* Methanolic extract of leaves of *Acacia nilotica* were more active against all five pathogens. It gives highest 24mm zone of inhibition (ZOI) against *Pseudomonas.* Chloroform extract of leaves of *Acacia nilotica* only active against *Staphylococcus aureus.* It gives 13mm zone of inhibition (ZOI) against *Staphylococcus aureus.* Water extract of leaves of *Acacia nilotica* only active against *Staphylococcus aureus.* Water extract gives highest 16mm zone of inhibition (ZOI) against *Staphylococcus aureus,* whereas Methanolic and Chloroform extract gives zone of inhibition (ZOI) 15mm and 13mm.

Table 2 shows the results of phytochemical analysis of leaves extract (Powder, Chloroform, Methanol and Water) of *Acacia nilotica*. Result from phytochemical analysis using powder extracts confirmed the presence of Alkaloids, Phenol, Tannin, Flavonoid and Quinones. whereas Chloroform extract confirmed the presence of Alkaloids, Phenol, Protein and Quinones. Methanol extract confirmed the presence of Alkaloids, Glycoside, Seponins, Phenol, Tannin and Quinones while Water extract confirmed the presence of Alkaloids, Glycoside, Seponins, Phytosterol, Phenol, Tannin and Protein.

Table 3 shows results of Thin layer chromatography (TLC).

Table 1: Antimicrobial activity of leaves extract of *Acacia nilotica*

Tested microorganisms	Diameter of inhibition zone (mm)		
	Methanol Extract	Chloroform Extract	Water Extract
Escherichia coli	23	00	00
Pseudomonas	24	00	00
Enterobacter	22	00	00
Staphylococcus aureus	15	13	16
Candida albicans	23	00	00

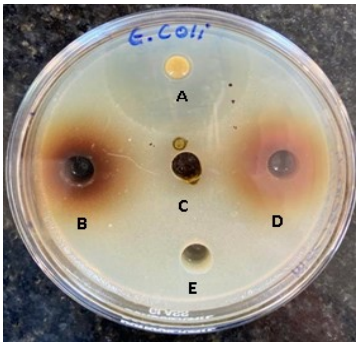

Figure 1: Antimicrobial activity of crude leaves extract of *Acacia nilotica* against *Escherichia coli* (A)Ampicillin (Positive control); (B)Water extract; (C)Chloroform extract; (D)Methanol extract; (E)DMSO (Dimethyl sulphoxide) (Negative control)

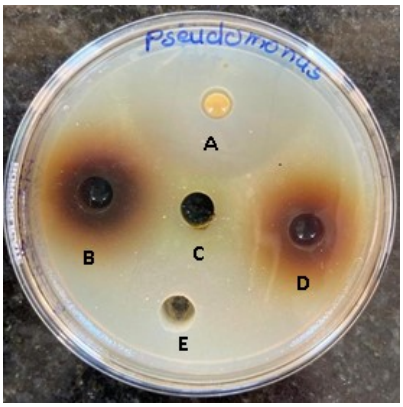

Figure 2: Antimicrobial activity of crude leaves extract of *Acacia nilotica* against *Pseudomonas*
(A) Ampicillin (Positive control); (B) Water extract; (C) Chloroform extract; (D) Methanol extract; (E) DMSO (Dimethyl sulphoxide) (Negative control)

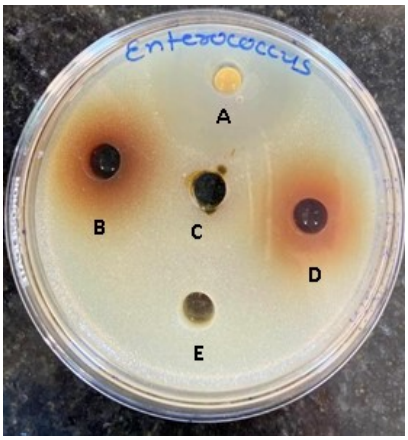

Figure 3: Antimicrobial activity of crude leaves extract of *Acacia nilotica* against *Enterobacter*
(A) Ampicillin (Positive control); (B)Water extract; (C) Chloroform extract; (D) Methanol extract; (E) DMSO (Dimethyl sulphoxide) (Negative control)

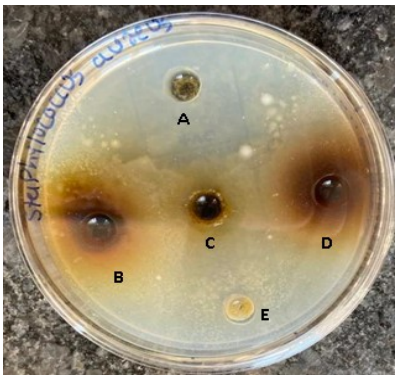

Figure 4: Antimicrobial activity of crude leaves extract of *Acacia nilotica* against *Staphylococcus aureus*
(A) Ampicillin (Positive control); (B) Water extract; (C) Chloroform extract; (D) Methanol extract; (E) DMSO (Dimethyl sulphoxide) (Negative control)

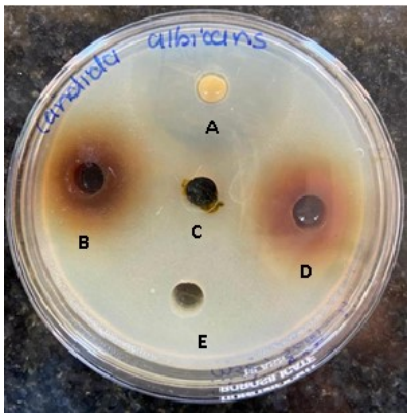

Figure 5: Antimicrobial activity of crude leaves extract of *Acacia nilotica* against *Candida albicans*
(A) Ampicillin (Positive control); (B) Water extract; (C) Chloroform extract; (D) Methanol extract; (E) DMSO (Dimethyl sulphoxide) (Negative control)

Table 2: Preliminary phytochemical analysis of leaves extract of *Acacia nilotica*

S. No	Components	Powder	Chloroform	Methanol	Water
1	Alkaloids	+	+	+	+
2	Carbohydrate		-	-	-
3	Glycoside	-	-	+	+
4	Seponins	-	-	+	+
5	Phytosterol	-	-	-	+
6	Phenol	+	+	+	+
7	Tannin	+	-	+	+
8	Flavonoid	+	-	-	-
9	Amino acid	-	-	-	-
10	Protein	-	+	-	+
11	Coumarin	-	-	-	-
12	Quinones	+	+	+	-

Key: (+) = present (-) = absent

Table 3: RF value

S. No	Extracts	RF value
1	Methanol	0.693
2	Chloroform	0.842
3	Water	0.798

CONCLUSION

Since ancient times, *Acacia nilotica* has been used to treat wide range of disease. Experimental studies have proven its antihypertensive, antidiabetic, antispasmodic, antibacterial, antifungal activity, antiplasmodic, antioxidant, antiviral activity. The present study has also confirmed the antimicrobial activity of Methanolic, Chloroform and Water extract of *Acacia nilotica* leaves against the selected human pathogenic bacteria. *Acacia nilotica* is a strong source of phytoconstituents having wide range of pharmacological properties. From the results of the phytochemical analysis, it was discovered that the leaves of *Acacia nilotica* contain various phytochemicals constituents in different solvents (Methanol, Chloroform, Water and Powder). The current study shows the presence of various phytochemical constituents i.e. alkaloids, glycoside, seponins, phytosterol, phenol, tannin,

flavonoid, protein and Quinone's. The phytochemicals analysis of the medicinal plant are important and have commercial interest in both research and pharmaceuticals companies for the manufacturing of the new drugs.

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