

Original Research Article

Efficacy of *Acacia nilotica* Extracts Towards Microbicidal Activity against Pathogens

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ABSTRACT

Keywords

Acacia nilotica, phytochemical properties, Bioactive constituents, Antimicrobial, Antioxidant

Human knowledge is wide as long as he can get an adaptation in new environment; the traditional medicine involves the use of different plants or the bioactive constituents different plants and this was done long time ago based on the history of human being. This study provides the health application of *acacia nilotica*, based on its secondary metabolites which are responsible for medicinal activities. Phytochemical analysis of *acacia nilotica* plant confirm the presence of various phytochemicals saponins, terpenoids, steroids, anthocyanins, coumarins and tannins, The results suggest that the phytochemical properties for curing various ailments and possess potential antimicrobial, antioxidant and leads to the isolation of new and novel compounds.

Introduction

Since the starting of medicinal activities human being want to search new drugs for the present diseases and based on his daily food mainly composed of vegetables, leads to the discover of different medicinal plants. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries used traditional medicines, which have compounds derived from medicinal plants. Those plants used should be investigated to better understand their properties, safety and efficiency. (Arunkumar and Muthuselvam, 2009).

Medicinal plants are those plants which

show antimicrobial, antifungal, antiviral or insecticidal activities.

From the best known and used medicine shows the high level of usage of those plants and while traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations (Cowan *et al.*, 1999).

There are three ways in which plants have been found useful in medicine. First, they may be used directly as teas or in other extracted forms for their natural chemical constituents. Second, they may be used as agents in the synthesis of drugs. Finally, the organic molecules found in plants may be

used as models for synthetic drugs. These forms are achieved by two different ways herbalism and phytotherapy.

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (Lai and Roy, 2004).

Medicinal plants

The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization (WHO) estimates that 80 percent of the Asian and African countries presently use herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available. The annual global export value of pharmaceutical plants in 2011 accounted for over US\$2.2 billion.

Phytochemistry

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into primary metabolites such as sugars and

fats, which are found in all plants; and secondary metabolites compounds which are found in a smaller range of plants, serving a more specific function. For example, some secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination. It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs examples are inulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove. Toxic plants even have use in pharmaceutical development.

Acacia plant

Acacia known commonly as *Acacia*, thorn tree, whistling thorn, or wattle, is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae, described by the Swedish botanist Carl Linnaeus in 1773 based on the African species *Acacia nilotica*. Many non-Australian species tend to be thorny, whereas the majority of Australian *Acacias* are not. All species are pod-bearing, with sap and leaves often bearing large amounts of tannins and condensed tannins that historically found use as pharmaceuticals and preservatives.

The generic name derives from (*akakia*), the name given by early Greek botanist-physician Pedanius Dioscorides (middle to late first century) to the medicinal tree *A. nilotica* in his book *Materia Medica*. This name derives from the Greek word for its characteristic thorns. The species name *nilotica* was given by Linnaeus from this tree's best-known range along the Nile River.

Table.1 Taxonomical classification of *Acacia nilotica* species

KINGDOM	Plantae
SUBKINGDOM	Tracheobionta
SUPER DIVISION	spermatophyta
DIVISION	Magnoliophyta
CLASS	magnoliopsida
SUBCLASS	rosidae
ORDER	Fabales
FAMILY	Fabaceae
GENUS	<i>Acacia</i>
SPECIES	<i>nilotica</i>

Acacia nilotica is a shrub or tree belonging to the family Leguminosae. It is widely distributed in Kenya and is widely used for medicinal purposes in both human and veterinary medicine in resource-poor rural and urban households. The decoction of its stem barks is used against diarrhoea and eye problems in livestock, stomachache, malaria, coughs, and primary infection of syphilis, sterility, and pneumonia in human being (Kokwaro, 1976).

It is a low, branched tree with a more or less spherical crown. Black bark on stem becomes ash-grey to light brown on the branches, bearing small, short, sharply hooked spines in pairs. It has a shallow but extensive root system radiating from the crown, allowing the plant to exploit soil moisture and nutrients from a large volume of soil. The roots rarely penetrate more than 1 m. leaves characterized by 2 pairs of pinnulae, each with a single pair of leaflets. Leaflets elliptic 0.6-2 cm long and 0.6–1.2 cm wide, glabrous and highly coloured beneath.

Today, traditional medicinal practices form an integral part of complementary or alternative medicine. Although their efficacy and mechanism of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their chemical constituents. The aim of this study is to assess the phytochemicals present in *Acacia nilotica* and examine their anti microbial effects.

Materials and Methods

Sample collection

Fresh leaves of *Acacia nilotica* were collected in Tamil nadu, Tanjavur district, in garden RAJA SARFOGI College.

The plant materials were dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were ground well using mechanical blender into fine powder labeling for future use.

Preparation of plant extracts

Crude plant extracts were prepared by Soxhlet extraction method. About 20 g of powdered plant leaves was uniformly packed into a thimble and extracted with 180 ml of different solvents separately.

Solvents used were methanol, ethanol and acetone. The process of extraction till the solvent in siphon tube of an extractor became colorless. After that the extracts were taken in beakers and kept on a hot plate and heated at 30–40°C till all the solvent got evaporated. Dried extracts were kept in a refrigerator at 4°C for their future use in phytochemical analysis.

Antibacterial study

Micro organisms

In this study both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria were used to determine antibacterial activity of different alcoholic extracts of plant *Acacia nilotica*.

Bacteria broth was prepared by dissolving 1.3 gr of nutrient broth in 100 ml of distilled water. Then, took loop full of bacteria culture from the slant and inoculate bacteria into broth medium. Incubation took place for 18-24 hrs at 37°C.

Determination of antibacterial activity

During this study antibacterial activity of *Acacia nilotica* extracts were carried out by a modified well agar method. Mueller Hinton agar plates were swabbed with 24 hrs old broth culture of selected bacteria. Consequently, using sterile borer, well of 0.6 cm diameter was made into each Mueller Hinton agar 4 wells were made and 40 micro liter of each extract was filled into the well.

The control antibiotic (Tetracycline) was used to compare each extract activity, and then the plates were incubated for 24 hrs at 37°C. Results were recorded by measuring the diameter of inhibitory zone by using a transparent meter rule at the end of 24 hrs.

Antifungal study

Micro organism

For this study, fungal strain, *Aspergillus niger* was used to determine antifungal activity of different extracts of plant *Acacia nilotica*.

Potato dextrose broth was prepared by dissolving 3.9g of potato dextrose broth into 100 ml of distilled water. A loop full of fungal culture from the slant was inoculated in broth medium and then incubated for 48 hrs at 37°C.

Determination of antifungal activity

In study, antifungal activity of *Acacia nilotica* extracts was carried out by a modified well agar method. Mueller Hinton agar plates were swabbed with 24 hrs old broth culture of selected fungal strain (*Asperigullus niger*). Consequently, using sterile borer, well of 0.6 cm diameter was made into each Mueller Hinton agar. Four wells were made and 40 micro liter of each extract was filled into the well.

The control antibiotic (Clotrimazole) was used to compare each extract activity, and then the plates were incubated for 24 hrs at 37 °C. Results were recorded by measuring the diameter of inhibitory zone by using a transparent meter rule at the end of 24 hrs.

Phytochemical analysis

All the extracts of powder of plant sample

were subjected to qualitative test for the identification of various plant constituents.

Test for alkaloid

a. Mayer's test: 0.5 ml of each plant extract was treated with Mayer's reagent; an appearance of yellow color indicates the presence of alkaloid.

b. Dragendroff's test: 0.5 ml of each plant extract was treated with Dragendroff's reagent (potassium bismuth iodide). An orange or orange red precipitate is formed.

c. Wagner's test: 0.5 ml of each plant extract was treated with Wager's reagent gives a brown or reddish brown precipitate.

Test for flavonoids

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.

Test for steroids (Liebermann test)

0.5 ml of the plant extract was mixed with 2 ml of acetic anhydride, 2 ml of chloroform followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

Test for saponins

5 ml of each plant extract was mixed in 80 % 1ml concentrated H₂SO₄. A layer of the green coloration was formed at the interface thus indicating a positive result for the presence of saponins.

Test for tannins

0.5 ml of plant extract was dissolved in 10

ml distilled water and filtered. 1% aqueous Iron chloride (FeCl₃) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test samples.

Test for glycoside

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. A more dense red precipitate indicates the presence of glycosides.

Test for terpenoid

5ml of aqueous extract of plant sample was mixed with 2ml of CHCl₃ in a test tube and 3ml of concentrated H₂SO₄ is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Antioxidant activity assay

To determine the reducing power assay of plant sample Yildirim *et al.* (2001) method was used.

Reagents required

Phosphate buffer, potassium ferric cyanide, trichloro acetic acid and ferric chloride.

Method

Different concentration of plant extract was mixed with phosphate buffer (2.5 ml 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000

rpm for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5ml, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicates stronger reducing power. The activity was compared with ascorbic acid standard.

Calculation

Percentage scavenging activity =

$$\frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorbance of the

control A_{test} is the absorbance in the presence of the sample.

Results and Discussion

The present study was carried out on antimicrobial and phytochemical screening of ethanolic extracts, methanolic extracts and acetone extract of *Acacia nilotica*.

Phytochemical analysis

The phytochemical analysis of leaves extract was carried out and was shown in the table 2.

Table.2 Preliminary phytochemical analysis

S. No	Phytochemical Constituents	Name of the Test	Acetone	Ethanol	Methanol
1	Alkaloid	Mayer’s test	-	+	+
		Dragendroff’s test	+	+	+
		Wagner Test	+	+	+
2	Flavonoids	Ammonia test	-	-	-
3	Steroids	Libermann’s test	+	+	+
4	Saponin	H ₂ SO ₄	+	+	+
5	Tannin	Lead Acetate	-	-	-
6	Glycoside	Fehling Solution A &B	-	-	-
7	Terpenoid	CHCl ₃ & Conc. H ₂ SO ₄	+	+	+

+ Present; - Absent

Antibacterial activity

The bacteria culture of *E. coli* and *staphylococcus aureus* in petriplates were incubated along with were checked for growth inhibition zones of organism after 24 hrs, the antibacterial activity of ethanolic, methanolic and acetone extracts of plant *Acacia nilotica* was studied and presented in table 4.

Antibacterial activity of dried leaves extract

and their efficiency were quantitatively assessed using agar well diffusion methods by measuring the diameter of growth of inhibition zone.

The present study indicates that the ethanolic extract of *Acacia nilotica* significantly suppress the growth of selected bacteria. The ethanolic extract of *Acacia nilotica* was most active against the microorganisms *Bacillus subtilis* and *Escherichia coli*. The maximum inhibition

zone was obtained in *E. coli* 26 mm and the minimum inhibition zone was methanolic extract found in *Staphylococcus aureus* which is 1 mm.

The comparison in strain shows that in gram negative *E. coli* the minimum zone of inhibition was observed on acetic extracts which is 23mm while the maximum was

25mm on methanolic extract, in gram positive *Staphylococcus aureus* the minimum zone of inhibition was observed on methanolic extract and was 13mm and the maximum one was 18mm on ethanolic extract. When compared to the ethanolic, methanolic and acetic extracts, ethanolic extract showed the highest zone of inhibition among the organisms.

Table.3 Antimicrobial activity

S.No	Name of organism	Zone of Inhibition(mm)			
		S	30%	50%	80%
1	<i>E. coli</i>	38	28	32	
2	<i>Staphylococcus aureus</i>	38	29	32	34
3	<i>A. niger</i>	30	20	25	28

Table.4 Minimum inhibitory concentration of the extracts against pathogens

S.no	Name of organism	MIC(mg/ml)		
		Ethanolic extract	Methanolic extract	Acetone extract
1	<i>E. coli</i>	2.5	9	4
2	<i>Staphylococcus aureus</i>	5	10	7
3	<i>A.niger</i>	3	10	8

Table.5 *In-vitro* antioxidant activity of plant extract by reducing power scavenging activity

Sample Extracts	Inhibition values in %	
	0.5 ml	1.0 ml
30% Ethanolic extract	53.2	91.6
50% Ethanolic extract	54.3	69.3
80% Ethanolic extract	37.4	61.4

Fig.1 Antibacterial activity

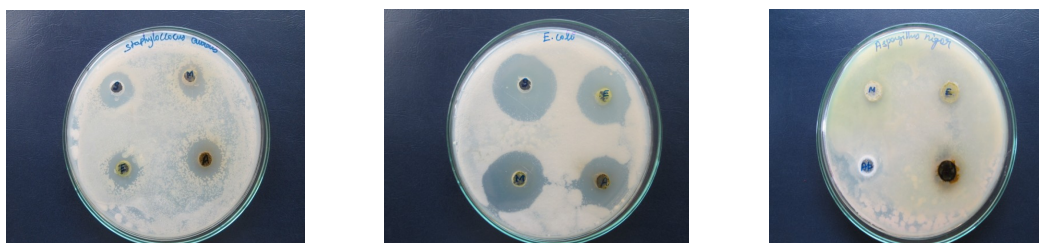


Fig.2 Ethanolic extract with different concentrations

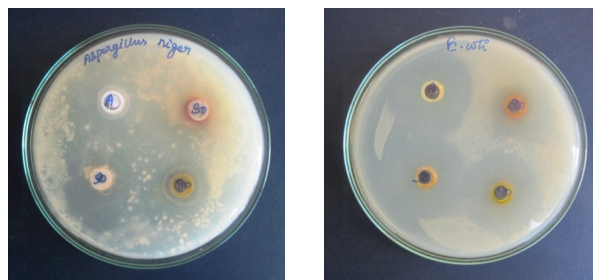


Fig.3 *In-vitro* antioxidant activity by reducing power scavenging activity

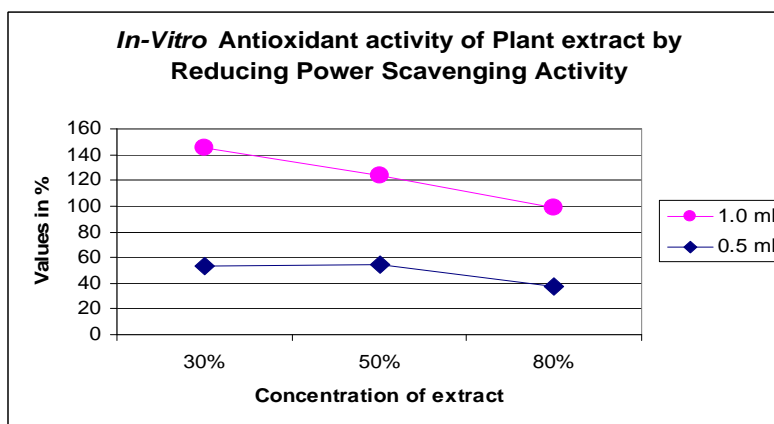


Table 4 shows minimum inhibitory concentration of the extracts against various pathogens used in this study. The MIC of ethanol extract was low (2.5mg/ml) as compared to other extracts (10 mg/ml). The lower MIC is an indication of high effectiveness of extract.

Antifungal activity

The fungal culture of *Aspergillus niger* in petriplate was along with the test were checked for growth inhibitions zone of organisms after 48hrs, the fungal activity ethanolic extract, methanolic extract, acetone extract of plants *Acacia nilotica* was studied.

The ethanolic extract of *Acacia nilotica* show the maximum zone of inhibition

against *Aspergillus niger* which is 13mm while the acetonic extract show the minimum one which is 10mm.

Antioxidant activity assay

Antioxidant is a substance that prevents or slows the breakdown of others substance by oxygen, they are chemical substances that donate an electron to the free radicals and convert it into harmless molecules.

Natural antioxidants that are present in different plants and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and plants contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the present study, we have evaluated the free radical scavenger activity

of ethanolic extract of *Acacia nilotica* with different concentrations.

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

In this study three extracts was evaluated (ethanolic, methanolic and acetone) for different phytochemicals, ethanolic extract showed the presence of more phytochemicals than others where showed the presence of alkaloids, steroids, saponnins and terpenoids. Medical important organisms were used in this study such as *E. coli* and staphylococcus aureus. Antibacterial activity of extracts and their efficiency were assessed using agar well diffusion methods by measuring the zone of inhibition diameter. The results showed that ethanolic extract is more powerful than other extracts, where it was most active against *E. coli* and *Staphylococcus aureus*.

The minimum inhibition zone was evaluated using ethanolic extract with different concentrations here 30%, 50% and 80% were chosen to be used. The 80% concentration showed maximum zone of inhibition in both *E. coli* and *Staphylococcus aureus* which is 34 mm.

When we compare all extracts ethanolic extracts showed high zone of inhibition against methanolic and acetone.

The culture *Aspergillus niger* was used for the antifungal activity test where the zone of inhibition was evaluated after 48 hrs, in all extracts here ethanolic extract showed high zone of inhibition which is 16 mm. Different

concentrations were used to evaluate the minimum zone of inhibition where 80% concentration of ethanolic extract showed high zone of inhibition (28 mm) and 30% showed the minimum one (20%).

In the present study the antioxidant activity was evaluated in ethanolic extract with different concentrations (30%, 50% and 80%) and different amount of extract (0.5 ml and 1.0 ml). Here 1.0 ml showed high antioxidant activity against 0.5 ml and 30% concentration showed high antioxidant activity 91.6%.

In this study where three assays were done (phytochemical analysis, antimicrobial and antifungal activity and antioxidant activity of *Acacia nilotica*) showed positive results to the following phytochemicals alkaloids, steroids, saponnins and terpenoids.

This study shows minimum inhibitory concentration of the extracts against various pathogens used in this study. The MIC of ethanol extract was low (2.5mg/ml) as compared to other extracts (10 mg/ml). The lower MIC is an indication of high effectiveness of extract.

It shows also antimicrobial effect on gram positive and gram negative bacteria as well as antifungal activity in addition it shows antioxidant activity.

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