



PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF ACACIA NILOTICA

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ABSTRACT

Acacia Nilotica is wide spread plant with useful traditional uses in Sudan. The acacia Nilotica was extracted with different polarity solvents (water, ethanol and petroleum ether). Phytochemical screening was done to different extracts, water and ethanol show more positive results (alkaloids, flavonoids, tannins, sterol and carbohydrate) compared to petroleum ether. The water and ethanol extracts suppressed the selected bacteria (E.Coli and S.aureus) while the petroleum ether show no inhibition zone in bacterial growth.

KEYWORDS: E.Coli and S.aureus.

1. INTRODUCTION

1.1. Background

Large sector of the Sudanese population use traditional medicine to meet their primary health care needs. In addition to being accessible and affordable, it is part of their belief systems. Often, traditional medicine provides the only available health care service to the population in many parts of the country.^[1]

What is traditional medicine?

The laity in the Sudan designates their healing corpus as *tibb* and the sophisticated among them qualify it as *tibb baladi*, local medicine. They understand *tibb* as a fine skill that requires knowledge, intelligence, and probably supernatural endowments such as magical powers and divine assistance. Incidentally, the word *tibb* also denotes magic. People would describe a sick person as *matbub*, bewitched and at the same time say *tabbab al-jarh*, treated the wound and *tabbab al-kasr*, set the broken bone. Due to beliefs in the supernatural causation of ill health, local medicine in the Sudan, like almost all other similar systems throughout the world, is integral to the systems describing cosmic relations-mystical, empirical or rational. Therefore, there are in the country as many systems of traditional medicine as there are ethnic or cultural groups.^[1]

1.2. Literature review

In study at June 2016 by Amira investigate the vitro antibacterial activity of acacia methanolic extract inhibitory effect against most of the tested. In study at September 1999 by N. K. Mustafa investigate the vitro

antimicrobial activity of acacia nilotica water and ethanol 80% extracts. And in previous study at November 2015 by Rwarinda U Angelo investigate the component of the acacia nilotica and the phytochemical screening test shows the presence of various component e.g. saponins, flavonoid, alkaloid and tannins.

1.2.1. Acacia nilotica

Acacia nilotica, known in Sudan as Garad, belongs to the family *fabaceae*. It is a native species of Acacia in Africa and the Indian subcontinent. Different parts of the tree are widely used in traditional medicine.^[2] Acacia species is considered as a rich source of Gallic and ellagic acid.^[3] It is a medicinally and economically important plant. Most of the acacias are of medicinal and health benefits to human being. For example, *Acacia nilotica* pods are used in treatment of wound (pods), malaria, sore throat (aerial part) and toothache (bark).^[4,5] while Gum Arabic is applied for kidney diseases treatment.^[6] Most of the acacias produce tannins. *Acacia nilotica* for instance, produces more than 20% tannins, especially the inner bark, which is used commercially for tanning and dyeing leather black in Sudan.^[7]

1.2.2. TAXONOMICAL CLASSIFICATION

Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Fabales

Family: Fabaceae
Genus: *Acacia*
Species: *nilotica*.^[8]

Synonyms– *Acacia nilotica* (Lam.), *Acacia scorpioides*, *Mimosa Arabica*, *Mimosa nilotica*, *Mimosa scorpioides*.^[9,10]

1.2.3. Common name

Afrikaans (lekkerruikpeul, ruikpeul); Amharic (cheba); Arabic (garad, sunut, sunt); English (prickly acacia, Egyptian thorn, babul acacia, Arabic gum tree, scented thorn, scented-pod acacia); French (gommier rouge, *Acacia de Cayenne*, *Acacia d'Arabie*, *acacia a gomme*); German (Gummi- Akazie, Arabische Akazie); Hindi (dauria, babla, kauria, godi, babul, telia, kikar, godi babul); Italian (*Acacia d'Egitto*); Ndebele (isanqawe, umtshanga); Somali (tuger); Swahili (mgunga); Tamil (karuvelum); Tigrigna (chea, gered chea, ghered); Tongan (nombe, mungnombie, mukoka); Trade name (babul); Tswana (motlabokgosi); Urdu (babar).^[11]

1.2.4 Morphological description

Acacia nilotica is a medium-sized, thorny tree, evergreen tree with a short trunk and having round spreading crown

with feathery foliage, found in the whole drier parts of India. *Acacia nilotica* is a single stemmed plant, grows to 15- 18 m in height and 2-3 m in diameter. The leaves (figure1.1) are fine and densely hairy with 3-6 pairs of pinnate consisting of 10-20 pairs of leaflets that are narrow with parallel margins and are rounded at the apex with a central midrib closely crowded.^[11] Thorns are thin, straight, light grey exists in axillary pairs (usually 3-12), 5-7.5 cm long in young trees. Flowers (figure1.2) bloom from June to September, and also in December to January. Flowers are globular heads, 1.2-1.5 cm in diameter of a bright golden yellow colour, develop either in axillary or whorly pattern on peduncles 2-3 cm long located at the end of branches.^[12] Pods (figure1.3) ripen in the months of May to June. Pods are 7-15 cm long, green and tomentose (when immature) or greenish black (when mature), indehiscent, deeply constricted between the seed giving a necklace appearance. Seeds are 8-12 per pod, compressed, ovoid, dark brown shining with hard testa.^[13] The gum exudes from the cuts in the bark in form of ovoid tears. The tears are glossy and marked with minute fissures and are brittle in nature. The colour of the gum varies from pale yellow to black. It is soluble in water.^[12]



Figure1.1: leaves



Figure1.2: flowers



Figure1.3: fruits

1.2.5. Habitat

Native: Botswana, Egypt, Eritrea, Ethiopia, India, Kenya, Mozambique, Namibia, Nigeria, Oman, Pakistan, Saudi Arabia, Sudan, Swaziland, Tanzania, Uganda, Yemen, Republic of, Zambia, Zimbabwe.^[14]

Exotic: Antigua and Barbuda, Australia, Bahamas, Barbados, Cape Verde, Cuba, Dominica, Dominican Republic, Grenada, Guadeloupe, Haiti, Iran, Iraq, Jamaica, Martinique, Montserrat, Nepal, Netherlands Antilles, Puerto Rico, South Africa, St Kitts and Nevis, St Lucia, St Vincent and the Grenadines, Trinidad and Tobago, Vietnam, Virgin Islands (US), Zanzibar.^[14]

1.2.6. Ethnomedicinal uses

Acacia nilotica is a pioneer species, relatively high in bioactive secondary compound and are important for a variety of functions is economically used as a source of tannins, gums, timber, fuel and fodder.^[15] *Acacia nilotica* plant is therapeutic used as anti-cancer, anti-tumors, antiscorbutic, astringent, anti-oxidant, natriuretic, antispasmodial, diuretic, intestinal pains and diarrhea, nerve stimulant, cold, congestion, coughs, dysentery, fever, hemorrhages, leucorrhea, ophthalmia and sclerosis.^[16] *Acacia nilotica* is the most important tree and almost all its parts are used in medicine, including leaves, bark, root, flower, pods, gum, etc.

Leaves: The leaves are astringent, tonic to the liver and the brain, antipyretic, enriches the blood. The tender leaves infusion used as an astringent and remedy for diarrhoea and dysentery.^[17,18]

Bark: Decoction of bark is largely used as an astringent douche in gonorrhoea, cystitis, vaginitis, leucorrhoea, prolapse of the uterus and piles.^[19]

Root: Powder of the root is useful in leucorrhoea, wound healing and burning sensation.^[20]

Flowers: It use as in diarrhoea and dysentery, premature ejaculation and seminorrhoea.

Pods: The pods are used for impotency, urino-genital disorder and in a dry cough. The seeds and leaf extracts are used for general body vigour.^[21]

Gums: The Gum is said to be very useful in diabetes mellitus.^[22]

CHAPTER 2: MATERIALS AND METHODS

2.1. Material and equipment

- *Acacia nilotica* fruits
- Solvents (ethanol 80%, DW and PE)
- Different species of bacteria (*S. aureus* and *E. coli*)
- Soxhlet
- Test tubes
- Petri dishes
- Separating funnel
- Measuring cylinder
- Pipits
- Beakers
- Neutral agar
- Mortar
- Pestle

2.2. Methods

2.2.1. sample collecting

The *Acacia nilotica* (fruits) were collected from Sudan at February 2017. It was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI). The fruits were air-dried under the shadow with good ventilation and then ground finely until their uses for extracts preparation.

2.2.2 Preparation of plant extracts

Crude plant extracts were prepared by Soxhlet extraction method. About 20 g of powdered plant fruits were uniformly packed into a thimble and extracted with 180 ml of different solvents separately. Solvents used were distilled water (DW), ethanol and petroleum ether (PE).^[23] 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.

2.2.3. Phytochemical screening tests

2.2.3.1. Test for alkaloid

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

2.2.3.2. 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.

2.2.3.3. Test for carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered.

Fehling's Test: Filtrate was hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

2.2.3.4. 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.

2.2.3.5. 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.

2.2.3.6. 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.

2.2.3.7. 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.

2.3. Antibacterial study

2.3.1 Micro organisms

In this study both gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria were used to determine antibacterial activity of different solvent 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.

2.3.2. 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 20 micro liters of each extract was filled into the well. 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.

CHAPTER 3: RESULTS

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

3.1. Results

3.1.1. Phytochemical analysis the phytochemical analysis of fruits extract was carried out and was shown in the table 1.

3.1.2. Antibacterial activity

The bacteria culture of *E. coli* and *S. aureus* in Petri dishes were incubated along with were checked for growth inhibition zones of organism after 24hrs figure 3.1, 3.2, the antibacterial activity of ethanolic, distilled water and petroleum ether extracts of plant *Acacia nilotica* was studied and presented in table 3.2 and table 3.3.

Table 3.1: phytochemical screening tests

	Alkaloids	Flavonoid	Saponins	Tannin	Cardiac Glycoside	Sterol	Carbohydrate
PE	+ve	+ve	-ve	±ve	-ve	+ve	-ve
Ethanol 80%	+ve	+ve	+ve	+ve	-ve	+ve	+ve
DW	+ve	+ve	+ve	+ve	-ve	-ve	+ve

Table 3.2: antibacterial activity test

	PE	Ethanol 80%	DW
<i>S. aureus</i>	-ve	+ve	+ve
<i>E. coli</i>	-ve	+ve	+ve

Table 3.3: zone of inhibition in antibacterial test

	PE	Ethanol 80%	DW
<i>S. aureus</i>	-	20 mm	30 mm
<i>E. coli</i>	-	27 mm	25 mm

Figure 3.1: *S. aureus*Figure 3.2: *E. coli*

CHAPTER 4: DISCUSSION

4.1. Discussion

The alkaloid and flavonoid had presence in each extract of *Acacia nilotica*, Saponins presented ethanol 80% and DW extracts while no trace of it in PE extract, Tannin presented with small amount in PE extract while it was presented with good amount in ethanol 80% and DW extracts, Sterol had a presence in PE and ethanol 80%

extracts while there is no trace in DW extract, Carbohydrates had appear in ethanol 80% and DW extracts while there is no trace in PE extract and Glycosides had negative results in all extracts. The ethanol 80% extract had the most positive results and the PE had most negative results. The glycoside had no trace with all extracts which is mean the *Acacia nilotica* doesn't contain cardiac glycoside. Antibacterial activity

of dried fruits extract and their efficiency were quantitatively assessed using agar good diffusion methods by measuring the diameter of growth of inhibition zone. The present study indicates that the ethanolic and DW extracts of *Acacia nilotica* significantly suppress the growth of selected bacteria. The DW extract of *Acacia nilotica* was most active against the microorganisms *S. aureus* and *E. coli*. The maximum inhibition zone was obtained in *S. aureus* (30mm) and the minimum inhibition zone was PE extract found in *S. aureus* and *E. coli* which is no inhibition zone. The comparison in strain shows that in gram negative *E. coli* the minimum zone of inhibition was observed on DW extracts which is (25mm) while the maximum was (27mm) on ethanolic extract, in gram positive *S. aureus* the minimum zone of inhibition was observed on ethanolic extract and was (20mm) and the maximum one was (30mm) on DW extract. When compared to the ethanolic, DW and PE extracts, ethanolic extract showed the highest zone of inhibition among the organisms.

Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antibacterial activity compared to water extract.^[24] Moreover, water is a better medium for the occurrence of the micro-organisms as compared to ethanol.^[25] From the observation of the phytochemical screening the result shows the presence of tannin, saponins and carbohydrates in polar solvents (ethanol 80% and DW) and that's due to its polar functional group and that's may be the reason of its antibacterial activity while the PE extract didn't show any antibacterial activity because the non-polar property which couldn't extract any of the active constituents.

4.2. CONCLUSION

The extract of *Acacia nilotica* with ethanol 80% shows presence of various phytochemical component and 20mm dimer of inhibition zone with gram positive bacteria *S. aureus* and 27mm dimer of inhibition zone with gram negative *E. coli*. The extractant of DW shows presence of phytochemical but it less than ethanolic extraction, the antibacterial activity test was better than the ethanolic extract with 30mm dimer of inhibition zone with *S. aureus* and 25mm dimer of inhibition zone with *E. coli*. The PE extract had the less amount of presence of phytochemical component and there is no antibacterial activity for this extractant.

4.3. Recommendation

The *Acacia nilotica* is useful medicinal plant with wide effective range I recommend to aware the community about its benefits and uses.

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