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# PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF THE EXTRACTS AND FRACTION OF *ACACIA GOURMAENSIS* A. CHEV. (FABACEAE-MIMOSOIDEAE)

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## ABSTRACT

The stem bark extracts and fraction of the plant *Acacia gourmaensis*, of the family Fabaceae, locally known as Anaka in Igala, Kaya yawo in Hausa, used in ethno-medicine for the treatment of convulsion, arthritis, jaundice, cancer, malaria, cough, rheumatism, lumbago, muscular/body pains, as well as in treatments of eye, ear, oral and pulmonary infections, management of snake bites and food poisoning in West African countries particularly Nigeria was investigated for its phytochemicals and antimicrobial activities. The preliminary phytochemical screening of the dichloromethane extract (DCM-E) revealed the presence of carbohydrates, fixed oils/fats, flavonoids, sterols and terpenes. The preliminary phytochemical screening of the methanol extract (MeOH-E) revealed the presence of alkaloids, carbohydrates, fixed oils/fats, flavonoids, glycosides, saponins, sterols, tannins and terpenes while the phytochemical screening of the ethyl acetate fraction (EA-F) also revealed the presence of carbohydrates, flavonoids, fixed oils/fats, sterols and terpenes. The antimicrobial activity was carried out using agar cup and plate diffusion method. The organisms tested were hospital isolates of Methicillin resistant *Staphylococcus aureus, Staphylococcus aureus, Bacillus subtilis, Corynebacterium ulcerans, Streptococcus pneumoniae, Campylobacter jejuni, Helicobacter pylori, Klebsiella pneumoniae, Proteus vettgeri, Candida albicans*, the result of the antimicrobial susceptibility test showed that both extracts and the fraction were active against five of the ten organisms with zones of inhibition ranging from 22 – 25 mm for MeOH–E, 23 – 26 mm for DCM-E and 26 – 28 mm for EA-F, while the zone of inhibition for the standard drugs ciprofloxacin 30µg/ml, Sparfloxacin 30µg/ml and fluconazole 30µg/ml were 32 – 37 mm, 32 – 37 mm and 0 – 35 mm respectively. The findings in this study showed that the extracts and fraction of the *A. goumaensis* contain phytochemical constituents with antimicrobial activity which necessitated or validated the use in traditional medicine.

**Keywords:** *Acacia gourmaensis*, antimicrobial, phytochemical, zones of inhibition
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## INTRODUCTION

Traditional herbal medicines are naturally occurring, plant derived substances with minimal or no industrial processing that have been used to treat illnesses within local or regional healing practices [1]. Traditional herbal medicines are getting significant attention in global health debates, in China, traditional herbal medicine played a prominent role in the strategy to contain and treat several diseases [2]. Ethnopharmacological uses of plants play very important role in the lives of Nigerian. It has been pointed out that plant continue to play a prominent role in the primary health care of about 80% of the world population [3, 4, 5]. *A. gourmaensis* belong to the genus acacia which include about 1400 species in subtropical and tropical Africa which include Nigeria, Senegal, Egypt and Mozambique as well as Asia from India to Buma according Ahmadu *et al.* [6].

Traditional herbal medicine and their preparations have been widely used for thousands of years in developing and developed countries owing to its natural origin and lesser side effects [7]. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations [8]. The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines such as aspirin (willow bark), morphine (from the opium poppy), digoxin (from foxglove), quinine (from cinchona bark) and pilocarpine (Jaborandi) according to Butler *et al.* [9]. Over the years there has been alarming report of multiple drug resistance in medically important strains of organisms which led to the development of more potent antibiotics such as 3rd and 4th generation Cephalosporins by Pharmaceutical companies. Many published reports have shown the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks of modern medicines for attainment of new active principles [4, 5, 10].

Morphologically, *Acacia goumaensis* is a woody plant found in open woodland and wooded grasslands of sub humid Africa. It is found throughout Africa and Australia [11]. *A. gourmaensis* is a small tree up to 25 ft high, often with straight stem and numerous short branches bole brown with small scales, bark of twigs, yellowish, peeling, and cream flowers [12]. The plant contains a variety of secondary metabolites such as Tannins, Alkaloids, Saponins and Flavonoids which have been found in vitro to have antimicrobial properties [6, 13, 14]. Literature review on the plant showed that the only information on the plant was the morphologic description according to Hutchinson and Dalziel [11], Musa Y.M. [15]. There is little or no information on the phytochemical constituents and antimicrobial uses of the plant, hence the rational for this study was to evaluate the phytochemical constituents and to authenticate the scientific bases for antimicrobial uses of *A. gourmaensis* in ethno or traditional medicines.

## MATERIALS AND METHODS

### Collection and identification of plant material

The stem bark, the leaves and branches of the plant were collected from Rijana village near Kontagora in Niger state by Mr. Gallah Shehu Umar of the Herbarium unit of the Department of Pharmacognosy and Drug Development Kaduna State University on 20th October 2018 where it was authenticated by comparing it with an existing voucher specimen number KASU/PCG/9882 and confirmed in the herbarium unit of the Department of Botany Faculty of life Sciences Ahmadu Bello University Zaria by comparing with an existing sample with a voucher number [01378] by Namadi Sunusi. The plant was air dried under the shade for 2 weeks and then the size reduced to a coarse powder using pestle and mortar and hence referred to as the powdered plant material.

### Processing of plant material and extraction procedure

The powdered stem bark (1.52 kg) was cold macerated stepwise exhaustively (one reagent to exhaustion before the next one) each time with 10.0 liters each of dichloromethane (DCM) and methanol (MeOH), they were both concentrated under the fans and were referred to respectively as dichloromethane extract (DCM-E) 39.24g (2.58% w/w) and methanol extract (MeOH-E) 50.00 (3.29% w/w). The methanol extract was partitioned with 2.5 liters of ethyl acetate to afford ethyl acetate fraction (EA-F) with 21.90g (1.38% w/w) yield and residual methanol extract (RMTN-E), the residual methanol extract was air dried and kept for further use.

### Phytochemical screening of the extracts and the fraction

The phytochemical screenings of the extracts and fraction of the plant *A. gourmaensis* for the various phytochemical groups were carried out according to those described by Sofowora [16] and Trease and Trease and Evan [5].

### Antimicrobial screening

### Test microorganisms.

The test microorganisms used for the study include: *M*ethicillin resistant *Staphylococcus aureus, Staphylococcus aureus, Bacillus subtilis, Corynebacterium ulcerans, Streptococcus pneumoniae, Campylobacter jejuni, Helicobacter pylori, Klebsiella pneumoniae, Proteus vettgeri, Candida albicans*. All the organisms were clinical isolates obtained from the Medical Microbiology Unit of Ahmadu Bello University Teaching Hospital Zaria, Nigeria.

### Susceptibility test

The antimicrobial activities of the extracts and the fraction of *A gourmaensis* were determined using some pathogenic microbes. 5.0 mg of the plant extracts and the fraction were weighed and dissolved in 10 ml of Dimethyl Sulphoxide (DMSO) to obtain a concentration of 5 mg/ml. Mueller Hinton agar was the medium used as the growth medium for the microbes. The medium was prepared according to the manufacturers' instructions, sterilized at $121^0$C for 15 minutes for bacteria, while for Fungi (*C. albicans)* Sabaraud's dextrose agar was used poured into sterile petri dishes and was allowed to cool and solidify and was incubated for 72 hours. Well diffusion method was used for screening the extracts. The sterilized medium was seeded with 0.1 ml of the standard inoculums of the test microbes Using a standard cork borer of 6 mm in diameter a well was cut at the center of each sealed with molten agar, inoculated medium. 0.1 ml of the solution of the extract of the concentration of 5 mg/ml was then introduced into the well on the inoculated medium. Incubation was made at 37$^o$C for 24 hours after which the plate of the medium was observed for zone of inhibition. The zone of inhibition was measured with a transparent ruler and the result recorded in millimeters (mm) according to Bauer *et al.* [8]

**Minimum Inhibition Concentration** (**MIC**)

The MIC of the extract was determined using the broth diffusion method. Mueller Hinton broth was prepared, 10 ml was dispensed into test tubes and was sterilized at 121ºC for 15 minutes, the broth was allowed to cool. McFarland's turbidity standard scale number 0.5 was prepared to give turbid solution. Normal saline was prepared 10 ml was dispensed into sterile test tube and microbe was inoculated and incubated at 37ºC for 6 hours. Dilution of the test microbe was done in the normal saline until the turbidity matched that of the Mc Farland's scale by visual comparison at this point, the test microbe has a concentration of about 1.5 x $10^8$ cfu/ml. Two-fold serial dilution of the extract was done in the sterile broth to obtain the concentration broth, 0.1 ml of the test microbe in the normal saline was then introduced into the different concentration, and incubation was made at 37ºC for 24 hours after which the test tubes of the broth were observed for turbidity (growth). The lowest concentration of the extract in the sterile broth which shows no turbidity was recorded as the Minimum Inhibitory Concentration [17-19].

**Minimum Bactericidal Concentration/Minimum Fungicidal Concentration** (**MBC/MFC**)

The MBC/MFC was carried out to determine whether the test microbes were killed or only their growth inhibited. Mueller Hinton agar was prepared, sterilized at 121ºC for 15 minutes, poured into sterile petri dishes and was allowed to cool and solidify. The contents of the MIC in the serial dilution was then sub-cultured onto the prepared medium, incubation was made at 37ºC and 25ºC for 48 and 72 hours respectively for bacteria and fungi, after which the plates were observed for colony growth, the MBC/MFC were the plates with lowest concentration of the extracts and fraction without colony growth.

## RESULTS

**Preliminary phytochemical screening of *A. goumaensis***

The results of preliminary phytochemical screening of the extracts and fraction of *A. gourmaensis* revealed the presence of the phytochemicals as shown in Table 1 below.

**Table 1:** Phytochemical profile of the extracts and the fraction of the *A. gourmaensis*

| Phytochemical Group | Extracts/fraction/inference(s) | | |
|---|---|---|---|
| | DCM-E | EA-F | MeOH-E |
| **Alkaloids** | - | + | + |
| **Anthraquinones** | - | - | - |
| **Carbohydrates** | + | + | + |
| **Fixed oils/fats** | + | + | + |
| **Flavonoids** | + | + | + |
| **Glycosides** | - | + | + |
| **Saponins** | - | + | + |
| **Sterols** | + | + | + |
| **Tannins** | _ | + | + |
| **Triterpenes** | + | - | + |

Key: + = Present, - = absent, DCM-E = dichloromethane extract, MeOH-E = methanol extract, EA-F = ethyl acetate fraction

The results of antimicrobial screening show that the extracts and the fraction were all active against five of the ten test organisms including: Methicilin resistant *Staphylococcus aureus*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Helicobacter pylori* and *Candida albicans*, details are as shown in Table 2 below:

**Table 2:** The zones of inhibition of the extracts and fraction of *A. gourmaensis*

| Test organism(s) | Zones of Inhibition (mm) | | |
|---|---|---|---|
| | **DCM-E** | **EA-F** | **MeOH-E** |
| MRSA | 25(S) | 27(S) | 25(S) |
| *Staphylococcus aureus* | 24(S) | 25(S) | 23(S) |
| *Bacillus subtilis* | 0(R) | 0(R) | 0(R) |
| *Corynebacterium ulcerans* | 0(R) | 0(R) | 0(R) |
| *Streptococcus pneumoniae* | 0(R) | 0(R) | 0(R) |
| *Campylobacter jejuni* | 23(S) | 25(S) | 23(S) |
| *Helicobacter pylori* | 26(S) | 28(S) | 24(S) |
| *Klebsiella pneumoniae* | 0(R) | 0(R) | 0(R) |
| *Proteus vettgeri* | 0(R) | 0(R) | 0(R) |
| *Candida albicans* | 23(S) | 26(S) | 22(S) |

**Table 3:** The zone of inhibition of the standard drugs against the test microorganisms

| **Test organism** | **Zones of Inhibition (mm)** | |
|---|---|---|
| | **Sparfloxacin (30µg/ml)** | **Fluconazole (30µg/ml)** |
| **MRSA** | 35(S) | 0(R) |
| ***Staphylococcus aureus*** | 32(S) | 0(R) |
| ***Bacillus subtilis*** | 37(S) | 0(R) |
| ***Corynebacterium ulcerans*** | 0(R) | 0(R) |
| ***Streptococcus pneumoniae*** | 30(S) | 0(R) |
| ***Campylobacter jejuni*** | 34(S) | 0(R) |
| ***Helicobacter pylori*** | 35(S) | 0(R) |
| ***Klebsiella pneumoniae*** | 33(S) | 0(R) |
| ***Proteus vettgeri*** | 34(S) | 0(R) |
| ***Candida albicans*** | 0(R) | 35(S) |

Key: MRSA= Methicillin-resistant *Staphylococcus aureus;* R = Resistant, S = Sensitive

**Table 4:** The MIC of the extracts and fraction of *A. gourmaensis* against the test microorganisms

| **Test organism** | **DCM- E (mg/ml)** | | | | | | **EA-F (mg/ml)** | | | | | | **MeOH-E (mg/ml)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Concentration** | 50 | 25 | 12.5 | 6.25 | 3.13 | 1.56 | 50 | 25 | 12.5 | 6.25 | 3.13 | 1.56 | 50 | 25 | 12.5 | 6.25 | 3.13 | 1.56 |
| **MRSA** | - | - | - | -* | + | + | - | - | -* | + | + | + | - | - | -* | + | + | + |
| ***S. aureus*** | - | - | -* | + | + | + | - | - | -* | + | + | + | - | - | -* | + | + | + |
| ***C. jejuni*** | - | - | -* | + | + | + | - | - | -* | + | + | + | - | - | -* | + | + | + |
| ***H. pylori*** | - | - | -* | + | + | + | - | - | -* | + | + | + | - | - | -* | + | + | + |
| ***C. albicans*** | - | - | -* | + | + | + | - | - | -* | + | + | + | - | - | -* | + | + | + |

Key: MRSA = Methicillin-resistant *Staphylococcus aureus;* Conc = Concentration

- = No colony growth; * = MIC; + = colony growth

**Table 5:** Minimum bactericidal (MBC)/Fungicidal (MFC) of the extracts and fraction against the test microorganisms

| **Test organism** | **DCM-E (mg/ml)** | | | | | | **EA-F (mg/ml)** | | | | | | **MeOH-E (mg/ml)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Concentration** | 50 | 25 | 12.5 | 6.25 | 3.13 | 1.56 | 50 | 25 | 12.5 | 6.25 | 3.13 | 1.56 | 50 | 25 | 12.5 | 6.25 | 3.13 | 1.56 |
| **MRSA** | - | - | -* | + | + | + | - | -* | + | + | + | + | - | -* | + | + | + | + |
| ***S. aureus*** | - | -* | + | + | + | + | -* | + | + | + | + | + | - | -* | + | + | + | + |
| ***C. jejuni*** | - | -* | + | + | + | + | -* | + | + | + | + | + | - | -* | + | + | + | + |
| ***H. pylori*** | - | - | -* | + | + | + | - | -* | + | + | + | + | -* | + | + | + | + | + |
| ***C. albicans*** | - | -* | + | + | + | + | -* | + | + | + | + | + | -* | + | + | + | + | + |

Key: MRSA = Methicillin-resistant *Staphylococcus aureus;* Conc = Concentration
- = No colony growth; * = MBC/MFC; + = colony growth

## RESULTS AND DISCUSSION

Plants are rich in a wide variety of secondary metabolite such as tannins, alkaloids, saponins and flavonoids which have been found *in vitro* to have antimicrobial properties [4, 5, 13]. The result of preliminary phytochemical screening carried out on the extracts and fraction of *A. gourmaensis* revealed the presence of carbohydrates, fixed oils/fats flavonoids, steroids and terpenes in the DCM-E, alkaloids, carbohydrates, fixed oils/fats, flavonoids, glycosides, saponins, sterols, tannins and triterpenes in the MeOH-E and alkaloids, carbohydrates, fixed oils/fats, flavonoids, glycosides, saponins, sterols, and tannins in the EA-F. These phytochemical constituents are known to possess many biological activities, the presence of these phytochemicals in *A. gourmaensis* may be responsible for its wide uses in ethno-medicine for the treatment of convulsion, arthritis, jaundice, cancer, malaria, cough, rheumatism, lumbago, muscular/body pains, as well as in treatments of eye, ear, oral and pulmonary infections, management of snake bites and food poisoning. Their presence may also be the reason for its uses as antibiotic anti-inflammatory and antioxidant in West African countries particularly Nigeria and other parts of the world where they are found., the presence of phytochemicals such as tannins are used in leather industries to treat hides and skins [4], also tannins a major constituent of canberry juice has for long been used to treat bacterial infection of the bladder [4, 18]. Saponins are used as starting material for the synthesis of steroids, oleanene, a saponin which occur in the root of Umbellifera is used in Chinese medicine in the treatment of hepato-biliary disorders and posses' anti-inflammatory property [4, 19], saponins are used in soap making industries/used in villages to poison streams or ponds in fishing [4, 5]. While some flavonoids have anti - tumor, antibacterial or antifungal property, some are used in domestic veterinary medicine, particularly in the form of ointment for treating dermal diseases [11]. The extracts and the fraction showed strong antimicrobial activity against Methicillin resistant *Staphylococcus aureus*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Heliobacter pylori* and *Candida albicans* but no activity against Vancomycin resistant *Bacillus subtilis, Corynebacterium ulcerans, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Proteus vettgeri. Staphylococcus aureus* is known to play a significant role in skin diseases including superficial and deep follicular lesion [1], *Staphylococcus aureus* is also implicated in causing sexually transmitted infections (STIs). So, the strong activity of the extracts and fraction on both the methicillin resistant *Staphylococcus aureus* and *Staphylococcus aureus* indicate strongly that the plant can be effective against skin and sexually transmitted infections.

It is important to note that the extracts and the fraction have activities against both bacteria and fungi making them to have a broad spectrum of activities. The zones of inhibition of the extracts and the fraction on the average was 23.40 – 26.20 mm was comparable with those of the standard drugs Sparfloxacin and Fluconazole 30.80 – 35.00 mm

which showed that the extracts and the fraction of *A. gourmaensis* can be effective against susceptible microorganisms (Tables 2 and 3). The MIC of the extracts and the fraction of *A. gourmaensis* averaged 12.50mg/kg (Table 4) while the MBC/MFC across the five sensitive bacteria and fungi averaged 12.50 – 25.00mg/kg for the DCM-E and 25.00 – 50.00mg/kg (Table 5). From the zones of inhibition results (Table 2) the extracts and the fraction of *A. gourmaensis* have strong activities against *MRSA* and *Staphylococcus aureus* meaning that *A. gourmaensis* can be used in the treatment of stubborn human sexually transmitted diseases (STDs) caused by these organisms which are usually difficult to treat with the modern/orthodox medicines, the strong sensitivity of *Candida albicans* means also that the plant can be used as anti- fungal agent for the treatment of fungal diseases caused by this organism. These plant extracts and fraction can also be used for the treatment of skin, hair and nail infections often caused by fungi. They can serve as good agents for the treatment of thrush, vaginitis and other conditions such as pulmonary and generalized infections including endocarditis caused by *Candida albicans* [4, 5]. These results further strongly validate the use of this plant *A. gourmaensis* in the treatment of a variety of diseases in traditional medicinal practices across Africa, Asia and other parts of the world.

## CONCLUSION

The stem bark of *A. gourmaensis* DCM-E, MeOH-E and EA-F have phytochemicals that have antimicrobial activities, this study validates the persistent use of the plant and other Acacia species in traditional medical practices in Africa, Asia and other parts of the world. Based on our current searching level this is the first time a study like this is carried out on *A. gourmaensis.*

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