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Phytochemical and Antimicrobial Analysis of *Acacia nilotica*

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ABSTRACT

This paper aimed at investigating the chemical constituents in Acacia nilotica that make it a unique plant remedy to spectrum of gastrointestinal ailments. To realize this quest, fruits and leaves samples of the plant were collected, washed, and then dried under ambient laboratory temperature. Further, the samples were crushed and ground to powder substances separately. 80% of aqueous ethanol was used to extract the bioactive compounds of each sample for phytochemical analysis. The results of the analyses indicated presence of Alkaloids, Flavonoids, Carbohydrates, Polyphenols, Sterols, and Saponins in both samples. Furthermore, antimicrobial analyses of the extracts were conducted to establish their efficacy on some selected gastrointestinal bacteria species. From the analyses, it was observed that A. nilotica fruit extracts had demonstrated tremendous sweep against the sampled bacteria species more than the leaves extract as observed from their zones of inhibition. From the results of the analyses, some recommendations were given.

Keywords: Phytochemicals, gastrointestinal bacteria, antimicrobial activity

Introduction

Since antiquity man has been struggling to overcome problems that have become treacherous to his life. These problems according to Millennium Project (2009) are called global challenges to humanity and there are fifteen prominent ones in the available database. Outstanding among them are poverty, diseases, global warming, water scarcity, overpopulation to mention but a few. Diseases which are seemingly the most teething threat amongst them have been crippling economic growth and educational development particularly in Africa (Rabinovich, 2002). Many factors have definitely been confirmed to contribute to the wide spread of diseases in Africa. The most influential are poverty, poor governance and inaccessibility to health facilities. As a result of the aforementioned, rural

people are compelled to opt for folklore medicines as alternative source of treatment to common diseases (Rout, Panda & Mishra, 2009). However, medicinal plant products are too crude to be taken in their entirety; they are yet proven to be effective in eliminating disease causing organisms (Muhammad & Shinkafi 2007). In addition, insensitivity of synthesized antimicrobial drugs couples with microbes resistance to drugs also contributed towards dependence on folklore medicines in the treatment of variety of diseases (World Health Organization-WHO, 2016).

Furthermore, the minimal side reactions of crude plant decoctions and their virtually low cost of purchase have encouraged masses of developing countries to consider them as alternative (Yunusa, *et al.*, 2009). The safety and efficacy of medicinal plants in different parts of the world have drawn the

attention of WHO to support for their integration into the Primary and Secondary Health Care System of every nation (Adesanya, 2005; El-mahmoud & Ameh, 2007). Although, folklore medicines are proven safe for consumption (Adesanya, 2005; El-Mahmoud & Ameh, 2007). WHO (2010) in a later development reported that African folklore remedies are the major cause of renal and hepatic damages. Perhaps, this might be related to the presence of poisons substances and heavy metals deposits in their tissues.

Folklore remedies are derived from all species of plant including grasses, shrubs, trees and underground vegetations. Generally, folk medicines are obtained from all parts of plants. The substances that act and effect medical remediation in tissues and organs of human beings are chemicals called secondary metabolites. The metabolites according to Doughari (2006) include flavonoids, terpenes, phenols, alkaloids, sterols, waxes, fats, tannins, gum and others.

Spectrum of diseases afflicts the population of Northern Nigeria and claims thousands of lives yearly. The most common amongst are gastrointestinal problems which are majorly caused by *Salmonella typhi*, *Escherichia coli* and *Shigella dysenteriae* (Yunusa *et al.*, 2009). In spite of the frequent use of synthesized drugs in urban health institutions, reasonable number of rural dwellers as a result of inaccessibility to health facilities has resorted to folklore drugs as only source of treatment to common diseases. *Acacia nilotica* is a popular plant used widely in the treatment of variety of diseases in Africa and India (Food and Agricultural Organization - FAO, 2017). *A. nilotica* was found to demonstrate powerful effect in the treatment of blood pressure and diabetes. In this connection therefore, FAO (2017) has reported that, taking 12 g of ground *A. nilotica* seed pod was found very effective in the treatment of diarrhea.

In this work, efforts were made to investigate the phytochemical substances in *A. nilotica* fruits and leaves. Further, antimicrobial assay of the extracts from the said parts was conducted on some species of bacteria that were confirmed to be the causative agents of gastrointestinal problems in human beings especially children.

Material and Methods

Collection and Identification of Samples

Fresh fruits and leaves of *A. nilotica* were collected in a wild bush in Katagum Local Government of Bauchi State, Nigeria. The plant's parts were taxonomically identified and authenticated by some experts in Plant Biology attached to Biology Department, Aminu Saleh College of Education, Azare, Bauchi State with voucher number 1873 and 1874 respectively. The voucher specimens were deposited at the Biology Laboratory of the School of Science, Aminu Saleh College of Education, Azare. The fresh parts were thoroughly washed and separately dried under ambient laboratory temperature and humidity for ten days. The dried parts were then ground, sieved and finally kept in cleaned air tight bottles under same the same laboratory condition (Nair, Kalariya & Chamda; 2004).

Extraction of the Samples

10 g each of powdered fruits and leaves of *A. nilotica* were respectively soaked in two conical flasks containing 100cm³ of 80% aqueous ethanol. The mixture in each flask was vigorously stirred, closed with a cork and left to stay for 24 hours. After this period, the mixtures were then filtered into other clean flasks respectively. The above procedure was repeated three times consecutively. The final filtrates obtained were thereafter concentrated using rotary evaporator. The crude extracts left after the evaporation process were kept in clean capped bottles under 4⁰ C until used (Koube, *et al.*, 2017).

Phytochemical Analysis of the Extracts

The crude extract of the samples were further subjected to secondary metabolite assay to confirm the presence of alkaloids, carbohydrates, flavonoids, polyphenols, saponins and sterols. Detailed procedure of the analysis is presented in Table 1.

Table 1: Procedure for the identification of phytochemicals in *A. nilotica* extract

Phytochemical	Description of Test
Alkaloids	Mayer's reagent was prepared by dissolving 1:3HgCl ₂ and 5kg KI IN 60 ML and 10 ml water respectively. The two solutions were combined and the resulting solution made up with water to 100ml. Addition of few drops of Mayer reagents to acidify solution of the extract produces white or yellow precipitate
Flavinoids	Magnesium powder and few drops concentrated HCl were added to an alcoholic solution of the extract. Formation of orange, pink or purple colour indicates presence of flavonoids.
Other polyphenols	Addition of few drops of 5% Ferric Chloride solution to ethanolic solution of the extract produces a brown precipitate indicating polyphenols is present.
Sterols	2mg of the extract was dissolved in 1ml chloroform and 1ml of concentrated H ₂ SO ₄ added to form two phases. Formation of red or yellow colour indicates presence of sterols'
Saponins	Because of their surface active properties, the presence of saponins was detected if there is production of foam which is stable for about 15 minutes after shaking an aqueous solution of the extract.
Carbohydrates	Molisch reagent contains 1% naphthol in 80% ethanol (solution i) and concentrated H ₂ SO ₄ (solution ii). Two to three drops of solution I was added to solution of the extract and then solution II was added without mixing to form an upper phase. Formation of purple ring in the interface indicates presence of carbohydrates.

Sources: Adapted from Muhammad and Shinkafi (2007).

Isolation and Identification of the sampled Bacteria

The isolation and identification of the bacterial species (*Salmonella typhi*, *Escherichia coli* & *Shigella dysenteriae*) used in this study were done at a government hospital in Bauchi State. The samples from which the isolation was made were obtained from patients identified suffering from gastrointestinal problems. The sampled bacteria were afterward sensitized using Diagnostic

Sensitivity Test Agar (DSTA) and incubated at 37⁰C for 24 hours (Doughari, 2006).

Anti-Bacterial Assay of the Extracts

3 mg each of the dried extracts was weighed and dissolved into 10ml ethanol in sterile capped bottles. The contents of bottles were then vigorously shaken to make concentrated solutions of the extract. Further, the solutions were separately poured onto Whatman No 1 filter paper discs in another sterile screw capped bottles. This was done to ensure that the paper discs were absolutely saturated with the extract solutions. After this, the bottles and content were kept and dried in an electrical oven. A pair of

Sterilized forceps was used in picking *A. nilotica* fruits extract impregnated paper discs from a screw capped bottle and placed onto the DSTA in a labeled Petri-dish carrying sample of *E. coli*, *S. typhi* and *S. dysenteriae* respectively. The same procedure was done to the paper discs impregnated with *A. nilotica* leaves extracts. The dishes were then incubated at 37⁰ C for 24 hours. The aim of the above mentioned steps was mainly to determine the antimicrobial efficacy of the extracts on the above bacterial species. The efficacy of the extracts was determined by measuring the diameters of the zone of inhibition around each extract impregnated paper disc. Variably, ethanol imbued paper discs were used as control under the same procedure and condition (Mukhtar & Ghor, 2012).

Results and Discussion

Phytochemical Analysis

The results of the phytochemical analysis are presented in Table 2. From the table, it is obvious that though the *A. nilotica* fruit extract has demonstrated presence of high quantity of secondary metabolites (alkaloids, flavonoids, polyphenols, Sterols and saponins), the quantity

of carbohydrates (another metabolite) was observed to be in a low amount. On the other hand, the result of the analysis also indicates that the presence of the secondary metabolites substances in leave extract was in very low quantity. In addition, the result reveals that flavonoid and saponins were absolutely absent in the extract.

Table 2: Result of the Phytochemical Analysis of the Sampled Extracts

Phytochemical	Fruit Extract	Leaves Extract
Alkaloids	++	+
Flavonoids	++	-
Other polyphenols	++	+
Sterols	++	+
Carbohydrates	+	+
++	Appreciable quantity	
+	Non-appreciable quantity	

Antimicrobial Activity of the Extracts

The efficacy of the extracts was observed by the diameters of zone of inhibition around each extract saturated paper disc on the DSTA in the labeled Petri-dishes. From the dishes, the *A. nilotica* fruit extract has demonstrated significant impact on the bacteria species by as observed by measuring the diameter of the zones of inhibition around each saturated disc in the medium containing the sampled species as indicated in Table 3.

Table 3: Antimicrobial Activity of *A. nilotica* Fruit extract on the Sample Bacteria

S/n	Bacterium Species	Zone of Inhibition	Conclusion
1.	E. coli	14 mm	The phytochemical and antimicrobial analyses of <i>A. nilotica</i> (Bagaruwa) have confirmed the assertion and belief that people attached to it in the treatment of several diseases including gastrointestinal diseases such as dysentery and diarrhea.
2.	S. typhi	19 mm	
3.	S. dysenteriae	21 mm	

The leaves extract on the other end had demonstrated sweeps around each impregnated paper disc in the medium containing the said

bacteria species as presented in Table 4. On the controlled side therefore, there were unnoticeable zones of inhibition around each ethanol saturated paper disc in the media containing *E. coli*, *S. typhi* and *S. dysenteriae* respectively.

Table 4: Antimicrobial Activity of *A. nilotica* Leaves Extract on the Sample Bacteria.

S/n	Bacterium Species	Zone of Inhibition
1.	E. coli	13 mm
2.	S. typhi	17 mm
3.	S.dysenteriae	15 mm

A. nilotica (Bagaruwa) is an important folklore tree found available in Africa, India and Australia. The tree's part when processed to concoction is used in the treatment of variety of infectious diseases (Venkateshwarlu, Santosh, Anasuri, Naik, Suma, Swapna & Pranitha 2011). Beside this, *A. nilotica* bark and seed pods are used in local leather industry. Its wood is of high quality and therefore it is used in making tools' handles and upholstery works. The presence of secondary metabolites in the plant's samples used in this study has corroborated the finding of Malviya, Sapna, Rawal, Swati, Kharia and Verma (2011) who reveal that *A. nilotica* is rich in alkaloids, flavonoids, sterols, and saponins. The result of the antimicrobial activity has shown that the plant's extracts are very efficient in combating and eliminating gastrointestinal bacteria and indeed in congruence with finding of Kabbash, Garbi, and Osman (2015).

Recommendations

Based on the findings of the study, the following recommendations were therefore given

1. Ethno-medicinal plants should be protected and sustained'
2. Establishment of ethno-gardens should be encouraged in schools and community at large.
3. Ethno-plants institutes should be established in different locations in Nigeria.
4. Severe penalty should be labeled on unauthorized cutting down of trees.
5. A course "conservation of natural resources" should be introduced in all educational institutions with purpose to inculcate spirit and value to natural environment.

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