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Phytochemical screeening and anti-bacterial analysis of the root extracts of *Maytenus senegalensis*

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Abstract
Extraction was carried out on the root of *Maytenus senegalensis* using water and ethanol by the maceration method of solvent extraction. Proximate analysis of the sample was determined using standard methods by Association of Official Analytical Chemist (AOAC). The extracts were screened for phytochemicals and antibacterial activity, using standard methods. The Proximate analysis showed moisture (11.63±0.02)%, ash (4.13±0.02)%, crude fibre (8.47±0.02)%, crude protein (14.57±0.07)%, crude fat (5.08±0.12)% and total carbohydrate (56.13±0.20)%. Both extracts showed the presence of saponins, tannins, Phenols, steroidal glycosides, terpernoids, alkaloids and steroids. Flavonoid and cardiac glycoside was observed only in the aqueous extract. The zones of inhibition ranges from 7.2mm to 14.3mm, showing antibacterial activity against both gram positive *Staphylococcus aureus* and gram negative *Escherichia coli*. The highest zone of inhibition was observed with the aqueous extract on the gram positive *Staphylococcus aureus.*

Keywords: phytochemical, *maytenus senegalensis*, proximate analysis, antibacterial, minimum inhibitory concentration, minimum bactericidal concentration

Introduction

Long before the emergence of modern medicine, our ancestors have been able to properly care for their health. Though there was no school to study and understand the mechanism and working of drugs, there was no laboratory to prepare and identify drugs. But through trial and error they were able to learn that certain plants could be used to treat diseases. Herbal preparations usually made from the roots, flowers, bark or other extracts were the only effective remedies available to our ancestors. Today 30 percent of conventional drugs are derived from plants. In spite of the development in modern medicine, more than 80 percent of the world's populations still rely on herbal medicine (wondimu *et al.*, 2007) [22]. Herbal medicine is as old as the history of man and spans all cultures. Medical plants have being useful in the development of new drugs and continues to play an invaluable role in drug discovery process (Cragg *et al.*, 1999) [3]. Herbal medicine have formed the basis of therapeutic use in developing countries but recently have also seen an increase in the herbal medications in the developed world as well simply because these herbs/plants are relatively cheap, easily available and their use are dependent on ancestral experience (Pooja *et al.*, 2014) [17].

Nigeria as a developing country, still consisting of some undeveloped forest, rich in medicinal plants has not been properly taking advantage of by the relevant authorities, hence left only to the rural dwellers and those that can't afford modern medical care. But with the recent studies focusing on the investigation of the traditional Nigerian medicinal plants which have resulted in the identification new sources of therapeutic agents, I believe is a call to all relevant authorities to look into traditional medicine because it's full of potential. (Pooja *et al.*, 2014) [17].

Many of these natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents or serve as the starting point in the development of modern medicines. Herbs are safe, less toxic, economical and a reliable key natural resource of drugs all over the world (Vapoorte *et al*, 1998).

Medicinal and healing properties of herbs are closely related to their chemical components which are classified into some major groups like alkaloids, essential oils, steroids, saponins, tannins etc. and all of them show various pharmacological activities (Aiyegoro *et al*, 2010) [1].

Proximate analysis

Proximate are used in the analysis of biological materials as it reveals the major constituents of such consumable goods. They are a good approximation of the contents of packaged consumable goods and serve as cheap and easy verification panels i.e. testing can be used to verify lots, but cannot be used to validate a food processor or food processing. Nutritional panels on the unite states are regulated by the FDA and must undergo rigorous testing to ensure the exact and precise content of nutrient in order to prevent a food processor from making unfounded claims to the public.

From an industrial standard proximate includes six constituents: Ash content, Moisture content, Crude Protein, Crude Fibre, Crude Fat, Carbohydrate (calculation).

Phytochemicals

Phytochemicals are biologically active, naturally occurring chemical compounds found in fruits, vegetables, grains, nuts, tea and seeds that promote human health and prevent diseases (Nyamar *et al.,* 2016). The therapeutic effects of these medicinal plants can justifiably be attributed to, among others, the phytochemicals the contain especially the flavonoids, alkaloids, sterols, terpernoids, phenolics acids, stilbenes, lignans, tannins and saponins. The abundance of scientific evidence indicates that such bioactive compounds have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property(Pooja *et al.*,2014) [17].

They are chemical compounds produced by plants, generally to drive away competitors, predators or pathogens. This phytochemicals are otherwise called secondary metabolites, because they are products of metabolism that are not necessary for the existence to the organism (plant) synthesizing it. Humans have been found to take advantage of this phytochemicals as they are useful for various purposes ranging from health, agriculture and industrial uses (Nyamar *et al.,* 2016).

Antibacterial

The treatment for bacterial infections is called antibacterial/antibiotics; hence substance having the ability to kill or stop the activities of bacteria is called an antibiotic. A bactericidal antibiotic kills bacteria directly while bacteriostatic prevents the growth or division of the bacteria (Scholar and Pratt 2000) [18].

Aim

The aim of the research is to determine the Phytochemical and antibacterial property of the root extract of *Maytenus senegalensis.*

Objectives of study

The objective of the research includes:

1. To Carry-out extraction from the root of *Maytenus Senegalensis*
2. To do a proximate analysis of the Root sample of Maytenus Senegalensis
3. To determine Some Phytochemical Constituents Of The root extract of *Maytenus Senegalensis*
4. To test for the antibacterial activity Of the root extract of *Maytenus Senegalensis*

Materials and Methods

Sample Collection

The sample of *Maytenus senegalensis* was collected from a forest in langtang north local government area of plateau State, Nigeria. The sample was collected in the month of September, 2018, and was identified by Mr J.J Azila a plant taxonomist with the federal college of forestry, Jos, Plateau State, Nigeria.

Sample Preparation

The root of *Maytenus senegalensis* collected from the forest were thoroughly washed with distilled water and air-dried at room temperature under shade for two weeks, after which it was grounded manually into uniform powder using a mortar and pestle. The sample was then sieved using a mesh, sealed in a Container, labeled and kept in polyethylene bag for extractions and proximate analysis. Proximate analysis was carried out on the prepared sample using Standard methods described by Association of Official Analytical Chemists (AOAC 2010). 50g and 80g of the sample were extracted, using 500ml water and 500ml of ethanol respectively, for 72 hours then filtered using whatman filter paper. The crude extracts were concentrated in a rotary evaporator after which they are evaporated to dryness on a water bath.

Sterilization of apparatus

All the glass wares used were sterilized using hot air oven at 106^0C for one and half hours. The water used in making the solution was sterilized in an autoclave at 121^0C for 15minutes.

Antibacterial Screening

The general standard methods for detecting and determining an invitro antibacterial activity includes: Plate diffusion, serial diffusion and streak test.

Phytochemical screening of the extracts of *Maytenus senegalensis*

Both extracts (Aqueous and Organic) were tested for the presence or absence of some secondary metabolites; Alkaloids, tannins, saponins, flavonoids, phenols, volatile oil, cardiac glycosides, steroidal glycoside, Terpenoids and Steroids, using standard procedures described by Trease and Evans (1989) [20].

Results and Discussion

Table 1: Result of Proximate analysis

S/n	Test	Result (%)
1	Moisture content	11.63 ± 0.024
2	Ash Content	4.13 ± 0.021
3	Crude protein	14.57 ± 0.074
4	Crude fat	5.08 ± 0.116
5	Crude fibre	8.47 ± 0.025
6	Total Carbohydrate	56.13 ±0.200

The proximate analysis of the root sample of *M. senegalensis* results as shown in Table 1 shows; moisture content, ash content, volatile matter, fixed carbon crude protein, crude fat, crude fibre and total Carbohydrates shows that *M. senegalensis* is rich in carbohydrate (56.13±0.200%), showing it can be used as a source of energy for animals, as well as contains a reasonable amount of fat (5.08±0.116%), its Protein content (14.57±0.074%) shows it can contribute to the protein need of animals and its crude fibre Fiber (8.47±0.025%), shows it can help digestion of food.

Table 2: Result of Phytochemical Screening

S/n	Phytoconstituents	Chemical Test	Water Extract	Ethanol Extract
1	Saponins	Distilled Water	+	+
2	Phenols	Ferric Chloride and Distilled Water	+	+

3	Flavonoid	Shinoda's Test	+	-
4	Alkaloid	Meyers	+	+
		Pitric	-	-
5	Tannin	Ferric Chloride	+	+
6	Volatile Oil	Dil. NaOH and Dil.HCl	-	-
7	Cardiac Glycoside	Acetic acid, ferric chloride and sulphuric acid	+	-
8	Steroidal Glycoside	Salkwiskis' Test	+	+
9	Terpenoids	Chloroform and Sulphuric Acid	+	+
10	Steroids	Chloroform + Conc. H2SO4	+	-
		Acetic Acid + Conc. H2SO4	-	+

KEY: (+) Present (-) Absent

The Phytochemical analysis results obtained from both the aqueous and ethanolic extracts showed that the both contain important phytochemicals with antibacterial activities The phytochemicals observed to be present in both extracts are; Saponins, phenolics, tannins, steroidal glycoside, terpene, alkaloids and steroids. Flavonoid and Cardiac glycosides are observed to be present in the aqueous extract only.

The phytochemicals, alkaloids, flavonoids, tannins, terpernoids and phenols have been observed to show antibacterial activities (Preshant, *et al.*, 2011)

The presence of alkaloid, glycosides saponins and phenols suggest its possible use as an antidiarrhoel in addition to its anti-bacterial use

Results of antibacterial Sensitivity Test

Table 3: Result of the zone of inhibitions of the aqueous extracts

Organism/ Concentration	100mg/L	50mg/L	25mg/L	12.5mg/L	6.75mg/L
Staphylococcus aureus(mm)	14.3	14.2	13.8	13.0	12.5
Escherichia coli(mm)	10.7	9.0	8.7	8.0	7.7

Table 4: Result of zone of Inhibition of the organic Extract

Organism/ Concentration	100mg/L	50mg/L	25mg/L	12.5mg/L	6.75mg/L
Staphylococcus aureus(mm)	13.5	11.0	10.0	9.50	8.0
Escherichia coli(mm)	12.6	10.0	9.2	8.0	7.2

The antibacterial analysis result in table 4.3, shows that both of the extracts; the aqueous (Water) and the organic (Ethanol) showed some level of inhibition on both of the organisms *Staphylococcus aureus* and *Escherichia coli*, showing that the extract of the root of *Maytenus senegalensis* has anti-bacterial properties. For example in the 100mg/L concentration, its region of inhibition is 14.3mm for *S. aureus* and 10.7mm for *E. coli* as compared to a lower region of inhibition observed in the organic extracts 13.5mm in *S. aureus* and 12.6mm in *E. coli.* This result is close to that observed by Emgba *et al.,* (2015), on the leaf of M. senegalensis using ethanol as solvent of extraction, *Staphylococcus aureus* 8mm at 31.25µg/ml. The antibacterial activity shown by different solvent extracts is due to the various phytoconstituents present in them, which acts alone or in combination to inhibit bacterial growth. The plant extracts were rich in flavonoids, tannins, terpenoids and alkaloids all of which are known for their antibacterial effects.

Table 5: Result of Minimum Inhibitory Concentration (MIC)

Extract/ Organism	*Staphylococcus aureus(mg/L)*	*Escherichia coli(mg/L)*
Aqueous Extract	25	50
Organic Extract	100	100

The minimum inhibitory concentration (MIC) which is the concentration required to stop or inhibit the growth of the bacteria as shown in table 4.4 shows that E. coli required higher concentrations to inhibit its growth; 50mg/L and 100mg/L for the aqueous and ethanolic extracts respectively. While *S. aureus* required slightly lesser concentrations of 25mg/L and 100mg/L for the aqueous and ethanolic extracts respectively.

Table 6: Result of Minimum Bactericidal Concentration (MBC)

Extract/ Organism	*Staphylococcus aureus(mm)*	*Escherichia coli(mm)*
Aqueous Extract	50	100
Organic Extract	100	100

The minimum Bactericidal Concentration (MBC) which is the actual concentration required to kill the bacteria was observed for *S. aureus* to be 50mg/L and 100mg/L for the aqueous and ethanolic extract respectively, while for *E. coli* it was observed to be 100mg/L for both the aqueous and ethanolic extracts.

Conclusion

From the result obtained in this research, it is evident that the use of the root of *Maytenus Senegalensis* in the treatment of bacterial infections and pains resulting from bacterial infections isn't in isolation but scientifically related. Its proximate analysis reveals a reasonable amount of dietary nutrients. Its Phytochemical screening reveals the presence of various Phytochemicals with therapeutic activities, like flavonoids, alkaloids, terpernoids, phenols and tannins which have been associated with antibacterial effect. However, more studies will be required to determine its nutritional content.

Recommendation

Further studies should be carried out on the root and other parts of *Maytenus Senegalensis* in order to determine its

effectiveness on other bacterial species, also more studies is required to determine its nutritional content and toxicity level.

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