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ANTIMICROBIAL ACTIVITIES OF BLACK PLUM (*VITEX DONIANA* SWEET) LEAVES AND STEM BARK EXTRACTS AGAINST SELECTED CLINICAL PATHOGENS

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Introduction

Medicinal plants are abundant source of antimicrobial molecules. Wide range of medicinal plant extracts are used to treat several infections as they have potential antimicrobial activity. Some of these bioactive molecules are screened and traded in market as raw material for many herbal industries (Renisheya *et al.*, 2011; Ugwoke *et al.*, 2017). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, etc. which have been found *in vitro* to have antimicrobial properties. Experts turned their concentration back towards obtaining advantages from medicinal plants after observing more side effects of synthetic drugs compared to their benefits (Bushra *et al.*, 2012;

Abstract

Antimicrobial activities of black plum (*Vitex doniana* sweet) leaves and stem bark extracts against selected clinical isolates. Black plum, scientifically known as *Vitex doniana* sweet, is the most abundant and widespread of the genus occurring in savanna region which belongs to the family Vebanacae. This research is aimed at determining the antimicrobial activity of *Vitex doniana* sweet leaves and stem bark extracts with the use of different solvents such as hot water, ethanol and acetone against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Candida albicans* respectively, the plant parts where collected at Ombi 2, College of Agriculture, Science and Technology, Lafia. Maceration technique[was used in the extraction of 40g plant part powdered samples. The inhibition zone diameter was determined using agar well diffusion method as a measure of the antimicrobial potency of the extract

Compared with standard gentamicin and distilled water as positive and negative controls respectively, which showed 16mm-0.00mm. The result obtained from the pH value ranges from 7.6 - 7.2 for both leaves and stem bark in the order of hot water, ethanol and acetone. The percentage yields for both leaves and stem bark in the same order of solvents range from 19.95% - 10.13% respectively. This result showed that the percentage yield was higher in hot water when compared with other solvents. The zone of inhibition for plant leaves in the same order ranges from 15.50mg/ml – 7.50mg/ml while that of stem bark ranges from 12.50mg/ml – 5.25mg/ml respectively, the result showed zone of inhibition for both plant parts, and there is no significant difference from the result to that of standard positive control. This study supports the claims by traditional health practitioners on the therapeutic use of *Vitex doniana* sweet. This work therefore recommends the use of *V. doniana* for the treatment of several health challenges which can also work against resistant strains of organisms. Further scientific investigation should be carried out to determine the toxicity and level of phytochemicals present in various plant parts.

Keywords: Index term: antimicrobial, *Vitex doniana*, solvents, inhibition zone diameter

Anze *et al.*, 2017). The use of plants for the treatment of diseases and maintenance of good health has been well researched (Aiwonegbe *et al.*, 2018). Kambba and Hassan (2010) reported that plants and plant-based products are the bases of many modern pharmaceuticals used today for the treatment of various ailments. According to WHO about 80% of the world populations depend on plants based medicine for their health care. It was also stated that the majority of the population in the developing countries (like Nigeria) still rely on herbal medicines to meet their health needs. Modern society is now embracing the use of plants and plant-based products to meet societal health needs due to the fact that indiscriminate use of commercial antibiotics commonly utilized in the treatment of infectious diseases has led to the development of multiple drug resistance with attendant adverse effect on the host (Gupta *et al.*, 2008). This emergence of pathogens resistant to antibiotics as a result of their excessive use in clinical and veterinary applications represents a serious public health concern (Keymanesh *et al.*, 2009).

For more than three decades now, the world has experienced pathogenic-resistant bacteria which have been causing major health problems even though

the pharmaceutical industries have produced large quantities of antibiotics. The resistance of bacteria and fungi to these drugs is becoming increasingly important (Lagnika *et al.*, 2012). This resistance has led to the search for plants with antibacterial and antifungal activity in recent years.

Black plum (*Vitex doniana* sweet) of the family *Verbanaceae* is a tree that grows in open woodland and savannah regions of tropical Africa; it is the most found species of the genus *Vitex*, the plant is found in West Africa, it produces fruits which are oblong in shape, with sweet taste and is edible. The fruit is green when unripe and changes to dark brown blackish brown when fully ripe, with the pulp surrounding a hard stone containing 1 – 4 seeds (Ali *et al.*, 2017). It is known by the local names: *Dinya* (Hausa); *Galbihi* (Fulani); *Orinla* (Yoruba); *Uchakoro* (Igbo); *Huruu* (Tiv), and *Atsitsi* (Jukun), *Itsu* (Kuteb) amongst others (Ubandoma *et al.*, 2018).

Vitex doniana sweet parts have numerous applications in traditional medicine. The tree is used as treatment of several disorders and diseases which include rheumatism, hypertension, cancer, anemia, and the plant is said to improve fertility and is used to treat jaundice, leprosy, and dysentery (Orwa *et al.*, 2009).

MATERIALS AND METHODS

Collection and processing of samples

The leaves, and Stem bark of black plum (*Vitex doniana* sweet) were collected according to the method adopted by (Binta and Rugayyah., 2013) from the Black plum tree. After authentication, samples were washed with clean running water to remove dirt and rinsed with distilled water. Samples were cut into smaller bits and dried for two-weeks before being pulverized into powdered form using sterile mortar and pestle in the laboratory as described by Aiwonegbe *et al.*, (2018). The powdered samples were stored in an air-tight container for further work.

Antimicrobial test

The pure cultures of *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans* were collected from the stock cultures. They were authenticated by the medical laboratory scientists of the same department. The organisms were resuscitated by plating on fresh Nutrient agar (media), for 24hours.

Broth Culture

Broth culture of the test organisms were prepared by plating the organism in test tube containing 10ml of nutrient broth and this will be incubated at 37°C for three(3) hours prior to the time for antimicrobial test (Dauda *et al.*, 2011).

RESULTS AND DISCUSSION

The table 1 result showed that extract with hot water has higher pH values when compared to ethanol and acetone extracts for both plant parts the result showed higher value for hot water on both plant parts, same for ethanol and acetone of leaves and stem bark extracts respectively.

Percentage yields of extracts of leaves and stem bark.

The amounts of yield of plant extracts were determined as shown in the table 2.

$$\text{Thus: \% yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

The table above showed the various percentage yields for plant leaves and stem bark extracts, where hot water extract showed the highest percentage yield followed by ethanol and acetone respectively,

The result above showed that hot water has the highest amount of yield followed by ethanol and acetone extracts respectively for both plant parts.

These when compared with the research carried out by Ejikem and Henrietta (2010) revealed the opposite which could be as a result of different prevailing environmental conditions in the places where the plant samples were cultivated and collected.

Inhibition Zone Diameter (IZD)

The inhibition zone diameter were used *in vitro* to determine the antimicrobial activities of different plant parts extracted using different solvents presented in chart and compared by solvent used as a measure to determine the potency of the plant part extracts as described by Ejikeme and Henrietta (2010).

The table 3 showed the various zones of inhibitions for plant leaves extract for each solvent used were hot water showed highest for both *S. aureus* and *P. aeruginosa*, while *S. typhi* and *C. albicans* were higher in ethanol.

The table 4 shows the antimicrobial effect of stem bark extract of different solvents where the IZD were higher in this order of hot water ethanol and acetone for both *S. aureus* and *P. aeruginosa* while higher in ethanol for both *S. typhi* and *C. albicans*. The solvents used has shown to have caused variation in their antimicrobial property.

Chart 1: Inhibition Zone Diameter of leaves extracts on test organisms.

Chart 1 above shows the comparative antimicrobial effects of plant leaves extracts using different solvents at different concentrations on selected test organisms.

Chart 2: Inhibition Zone Diameter of stem bark extracts on test Organisms.

Chart 2 above shows the comparative antimicrobial effects of stem bark extracts using different solvents at different concentrations on selected test organisms.

The Confirmatory tests of the pure cultures

The pure cultures of the test organisms obtained were authenticated and confirmed as shown in table 5 below

Antimicrobial activities of *Vitex doniana* sweet extracts were investigated using different parameters, and different extraction solvents were different yields of the extracts were obtained probably because of solvents used and the methods adopted. Cold maceration has generally been reported to give lower yield compared to other extraction solvents (Ibrahim *et al.*, 2001). The hot water extract gave slightly higher yield than the cold water probably because of higher solubility of the plant component in hot water. the results obtained reviewed that different solvents were used in the extraction process, it therefore indicates that for both leave and stem back extract, the percentage yield were higher in hot water, followed by ethanol least been the acetone solvent respectively, in order of 19.95%, 16.88%, and 12.63%, while stem extract shows 17.20%, 15.63%, and 10.13% respectively.

This result when compared to that obtained from (Ejikeme and Henrietta, 2010). It showed that ethanol has the highest percentage yield of extracts, followed by acetone while the least is hot water, the reason for these variations can be related to the geographical location of the plant and also the age of the plant used.

Secondly, the pH values for each plant extract were determined for both leave and stem back extracts, the result showed that hot water extract has the highest value followed by ethanol, and acetone in the order of 7.6, 7.2, 7.4, for leaves extract, while 7.5, 7.3, and 7.2, for stem extract respectively which makes the plant extracts basic and safe for human consumption.

Antimicrobial activity of the extracts on the test organisms in this study revealed inhibition of growth, though the susceptibility pattern of the test organisms to the extract was not uniform. The results are shown on Tables 3. Indicates that for bacteria used the hot water extracts showed the highest level of susceptibility, followed by ethanol and acetone extract in the order of 15.50, 12.50, and 10.50 (mg/ml) respectively for each bacteria isolate, the opposite was recorded with the fungi *C. albicans* which shows highest susceptibility with ethanol extract followed by hot water lastly acetone in the order of 10.50, 8.50 and 7.50 (mg/ml) respectively. These could be as a result of the fact that different organisms have

different growth requirements which were not readily provided by the solvent used during the extraction processes.

In addition similar result was also obtained from stem bark with hot water having the highest inhibition zone diameter followed by ethanol and acetone respectively in the order of 12.50, 10.45, 9.50, (mg/ml) while ethanol extract has the highest followed by hot water lastly and acetone in the order of 14.50, 9.50 and 5.25 (mg/ml) respectively.

Comparatively, the result revealed that both leave and stem bark of *Vitex donianan* extracts showed similarity in their antimicrobial potency irrespective of the solvent used.

Table 1. Shows the pH values of leaves and stem bark extracts using different solvents

Plant extracts	Solvents used	pH values
Leaves extracts	Hot water leaves	7.6
	Ethanol leaves	7.2
	Acetone leaves	7.4
Stem bark extracts	Hot water stem bark	7.5
	Ethanol stem bark	7.3
	Acetone stem bark	7.2

Table 2: Shows the yields of crude leaves and stem bark extracts using different solvents

Plant extracts	Extraction solvent	Actual yield (mg)	Percentage yield of plant extracts (%)
Leaves extracts	Hot water	7.98	19.95
	Ethanol	6.75	16.88
	Acetone	5.05	12.63
Stem bark extracts	Hot water	6.98	17.20
	Ethanol	6.25	15.63
	Acetone	4.05	10.13

Table 3: Shows the antimicrobial effect of leaves extracts using different solvents

Test Organism	Mean Inhibition Zone Diameter (mm)		
	Hot water	Ethanol	Acetone
<i>Staphylococcus aureus</i>	15.50	12.20	8.50
<i>Pseudomonas aeruginosa</i>	12.50	10.25	5.50
<i>Salmonella typhi</i>	10.50	12.50	8.50
<i>Candida albicans</i>	8.50	10.55	7.50

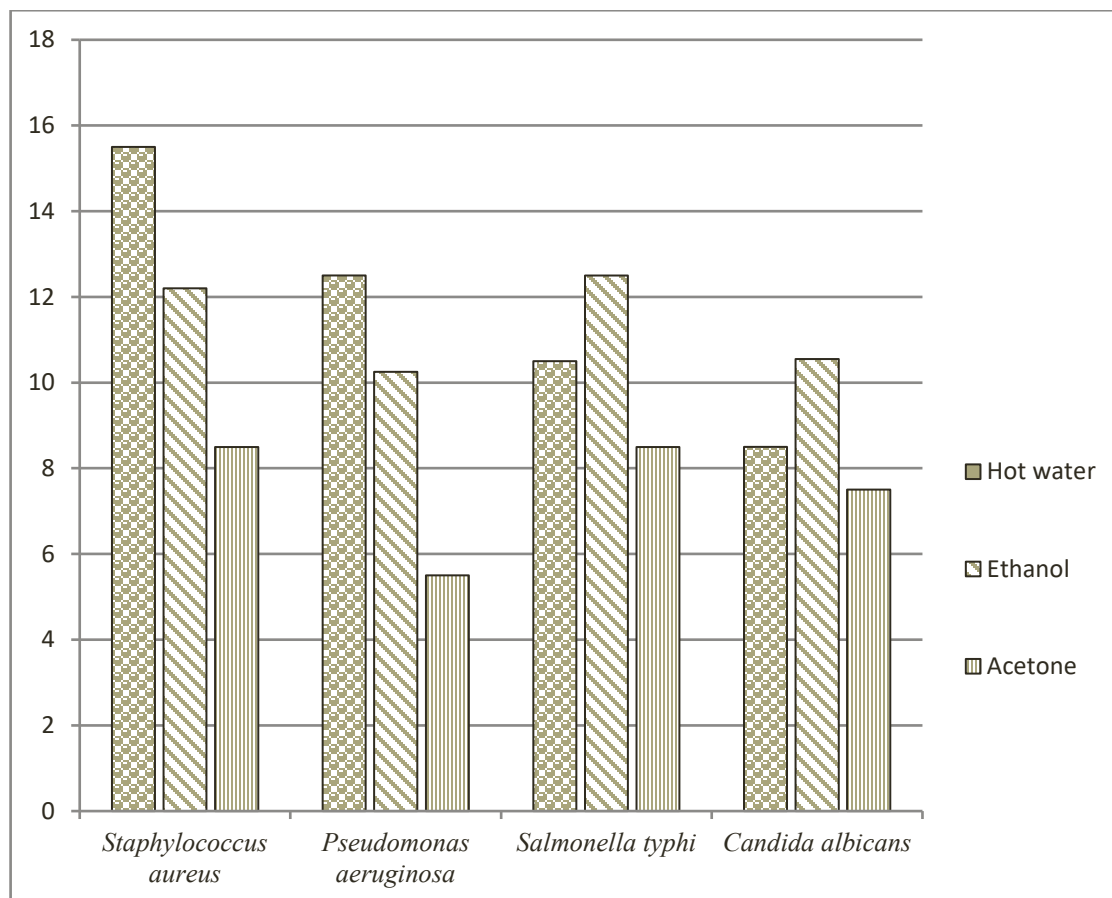
Table 4: Shows the antimicrobial effects of stem bark extracts using different solvents.

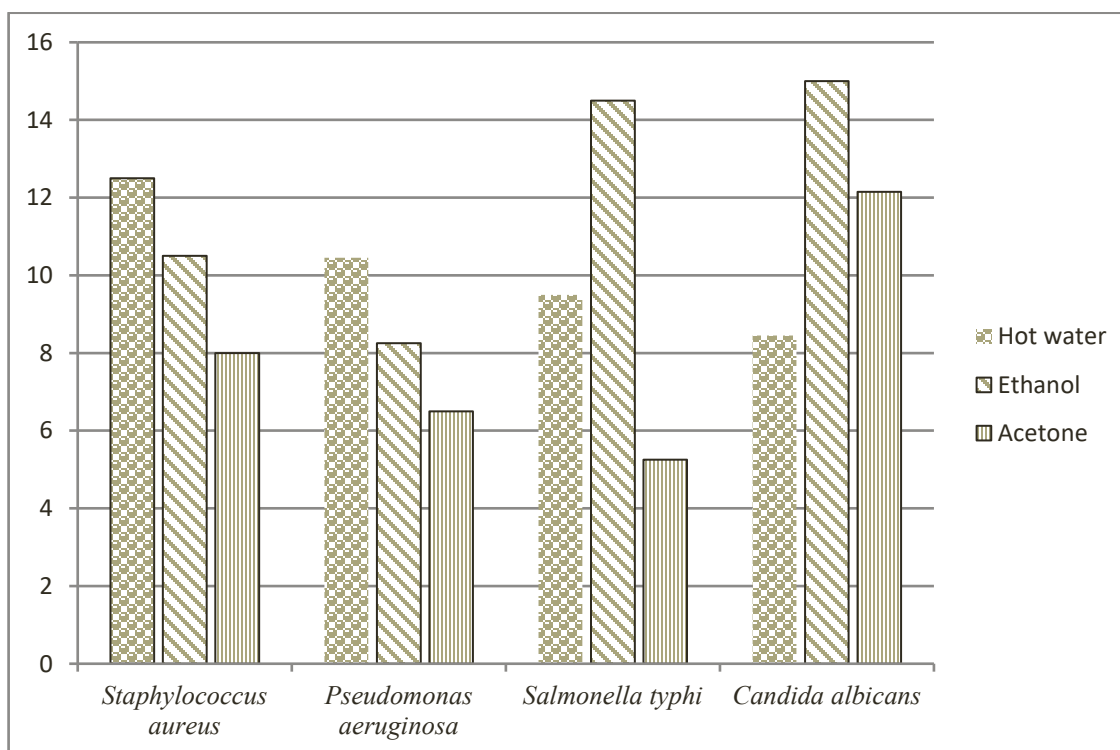
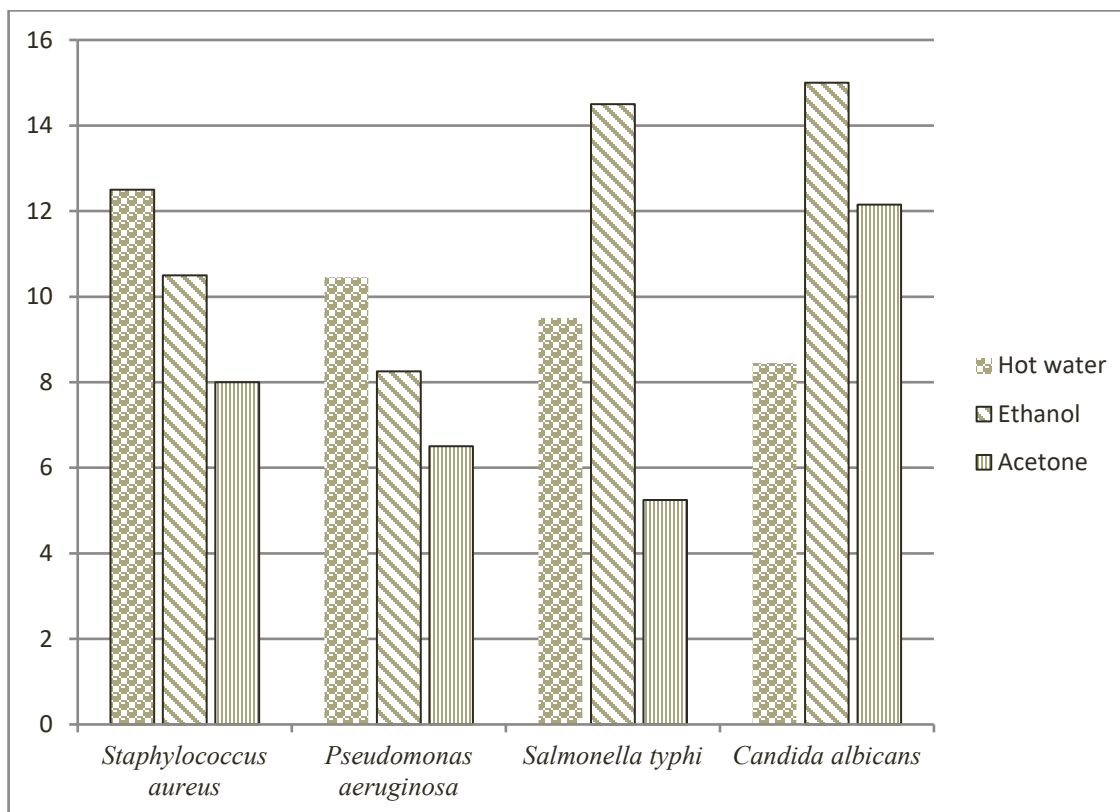
Test Organism	Mean Inhibition Zone Diameter (mm)		
	Hot water	Ethanol	Acetone
<i>Staphylococcus aureus</i>	12.50	10.50	8.00
<i>Pseudomonas aeruginosa</i>	10.45	8.25	6.50
<i>Salmonella typhi</i>	9.50	14.50	5.25
<i>Candida albicans</i>	8.45	15.00	12.15

Table 5: Biochemical test results of the test organisms obtained

Organisms	Gram reaction	Oxidase	Catalase	Coagulase	Citrate
<i>S. aureus</i>	+	-	+	+	+
<i>S. typhi</i>	-	-	+	+	+
<i>P. aeruginosa</i>	-	+	+	-	+
<i>C. albicans</i>	+	-	+	+	+

Key: (+) means Positive, **(-)** means Negative





Conclusion

In conclusion, this study was aimed to determine the antimicrobial potency of *Vitex doniana* sweet and to carry out a comparative antimicrobial assessment of leave and stem bark of the plant extracts. The result from this study will help in providing necessary information on one of the world most important public health issue, which is antibiotic resistance and could help in the search for new drugs especially those which can be used in treatment and management of several ailments.

Kambba and Hassan (2010) reported that plants and plant-based products are the bases of many modern pharmaceuticals used today for the treatment of various ailments. According to WHO (2001), about 80% of the world populations depend on plants based medicine for their health care.

The aqueous, ethanol and acetone extracts of *V. doniana* leaf and stem bark showed significant inhibition against the test organisms and the sensitivity increased with increase in concentration of the extracts. The study therefore provide basis for the use of this plant as remedy for rheumatism, hypertension, cancer and inflammatory diseases etc. And also justifies the folklore medicinal claim about the therapeutic value and antimicrobial effect of the plant extracts as has been shown by (Rugayyah and Muhammad, 2013).

This study therefore supports claims by traditional health practitioners on the therapeutics use of *Vitex doniana* sweet.

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