ISSN 2664-8075 (Print) & ISSN 2706-5774 (Online)
South Asian Research Journal of Applied Medical Sciences
Abbreviated Key Title: South Asian Res J App Med Sci

| Volume-4 | Issue-3 | May-Jun 2022 | **DOI: 10.36346/sarjams.2022.v04i03.002**

Original Research Article

Effects of Some Herbal Plants and Herbal Drink on Selected Micro-Organisms Using Ditch and Agar Methods

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Article History
Received: 06.04.2022
Accepted: 10.05.2022
Published: 15.05.2022

Abstract: This study was carried out to evaluate the effect of several herbal plants, Moringa, Usira and commercially sold African Bitters on selected micro-organisms. Adequate amount of fresh plants of *Moringa leaves* and Usira leaves were collected from a farm in Ekpoma Also, the commercial African bitters were purchased from herbal market in Ekpoma town, Edo State. The extract was prepared by weighing out 2g of the milled powdered powdered leaf of Moringa and Usira and adding 14ml of distilled deionized water and 10ml of 70% ethanol. The agar diffusion method and ditch method were employed for the sensitivity of the extracts on the bacterial isolates. The results on agar diffusion method showed that all the organisms isolated were resistant to moringa neat and distilled water extracts but were sensitive to ethanolic extract of moringa. Also, all the organisms were also resistant to usira neat and distilled water extract. However, *Candida albicans* was sensitive to ethanolic extract of usira. Furthermore, none of the organisms were sensitive to African biters in all the dilutions. The results on the ditch method showed that E. coli, *Candida albicans*, strep were sensitive while *Pseudomonas species* and *Klebsiella species* were not sensitive. It can be concluded that the extracts of *Moringa* and Usira (ethanol extract) inhibited the growth of bacteria isolates in a dose dependent fashion. Hence, it can be concluded that the leaf of *Moringa* and Usira can be a very good source for herbal drugs especially in using ethanol as a solvents.

Keywords: Moringa, Usira, Herbal, Plant, Ethanol, Extract.

INTRODUCTION

Plants are one of the most important sources of medicine. Plant derived compounds have been attracting much interest as natural alternatives to synthetic compounds (Kalimuthu *et al*., 2010). African plants, in particular medicinal plants constitute a rich but still largely untapped pool of natural products. Many countries from the developing world are still dependent on medicinal plants for treating the sick among them. The Nigerian climate favours a great array of plant species many of which have varied medicinal and antimicrobial potentials (Ezeifeka *et al*., 2004). Furthermore, Plants and derivatives of plant play a key role in world health and have long been known to possess biological activity (Abass, 2012). It is a known fact that thirty percent of all modern drugs are derived from plants and available evidence suggests that approximately 80% of Africans rely on traditional healthcare practitioners and medicinal plants for their daily healthcare needs (Burns, 2000; Johnson *et al.*, 2007; McKay *et al.*, 2007). Also, World Health Organization (WHO) estimates that almost 75% of the world's population has therapeutic experience with herbal remedies principally because of a belief that herbal remedies may have fewer side effects and can enhance the effects of conventional agents or be an

alternative treatment (Liu *et al.*, 2007; Desai *et al.*, 2009). The Nigerian antibiotics of interest include; Alligator pepper, *Moringa oleifera*, *Phyllantus amarus*, Bitter kola and Garlic.

Moringa oleifera is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for nutritional, medicinal and industrial uses. Powder from seed kernels of *Moringa* works as a natural coagulant and is used in the rural areas to clarify very turbid water (Gassenschmidt *et al*., 1995). Immature seeds of *M. oleifera* are used in recipes, the leaves are extensively used as vegetable in many parts of the world and the roots can be made into a condiment similar to horseradish (Prajapati *et al*., 2003). The therapeutic effects of *M. oleifera* include: antibiotic (Fahey *et al*., 2002; Haristoy *et al.*, 2005), anticancer (Guevara *et al.*, 1999; Bharali *et al.*, 2003), antiulcerogenic effects (Akhtar and Ahmad, 1995), analgesic (Rao and Ojha, 2003), antiurolithiatic and larvicidal activities (Bennett *et al.*, 2003).

Herbal bitters are usually poly-herbal liquid preparations which contain bitter herbs. There are other dosage forms, like capsules, tablets and tinctures which have been labeled by their manufacturers as bitters. They are commonly used as carminatives, aperitifs and to improve digestion. In Nigeria today, there is a preponderance of these products claiming to meet majority of the health need of the populace.

Usira (*Vetiveriazizanioides*) belongs to family Graminae/ Poaceae. It is one of the best refrigerant herbs that cools and calms the entire body and mind, with its influence spreading throughout the circulatory, digestive, reparatory, and urinary and nervous systems. It enjoys an important place among medicinal herbs in India since ancient times (Herbal cure India, 2015).

The development and wide spread of resistance of microorganisms to existing antibiotics calls for increased efforts in the development of new antibiotics for treatment of microbial infections and diseases. Although, there is a wide range of antibiotics for the treatment of these infections and diseases, the development of resistance to chemotherapeutic agents are increasingly becoming a serious and global problem. Globally, the last two decades has witnessed an unprecedented increase of drug resistance by pathogenic microorganisms as well as the appearance of undesirable side effects of certain antibiotics. Other limitations of modern chemotherapeutic drugs are their high cost and non-availability, especially in rural areas. As a consequence, it is necessary to search new organic molecules with antimicrobial activity, which in addition could be potential sources for starting materials for the semi-synthesis of new drugs (Akaochere *et al*., 2002).

Medicinal plants serve as the major source of medicines for treatment of various ailments in the primary health care for majority of the rural populace in Nigeria as in other parts of Africa (Iwu, 1993). These medicinal plants employed in traditional medicine represent potential sources of cheap and effective standardized herbal medicines (phytomedicine) and leads in the discovery of novel molecules for the development of new chemotherapeutic agents (Farnsworth and Morris, 1976; Sofowora, 1993).

MATERIALS AND METHODS

Adequate amount of fresh plants of *Moringa leaves* and Usira leaves were collected from a farm in Ekpoma, Esan West Local Government Area, Edo State between the hours of 7-9am. It was authenticated at the herbarium in the Department of Botany Ambrose Alli University, Ekpoma, Edo State. Also, the commercial African bitters were purchased from a herbal market in Ekpoma town, Edo State. Collection and buying of the materials tool place in the Month of August. The plants were preserved in a cool and dry place before use.

Fresh leaves of Moringa leaves and usira were properly washed using tap water and rinsed in distilled water. The leaves were dried in an oven at 60^{o}C to stop enzyme activity. They were dried to a constant weight and milled to a fine powder with the aid of a binatone blender. Two solvents were used for the preparation of the extract. The two solvents were distilled deionized water and 70% ethanol. The extract was prepared by weighing out 2g of the milled powdered powdered leaf of Moringa and Usira and adding 14ml of distilled deionized water. Also 2g of Moringa and Usira leaves added to 10ml of 70% ethanol.

The different test organisms were isolated from urine, throat swabs and nasal swab sample from the Irrua Specialist Teaching Hospital (ISTH), Irrua, Edo State. These test organisms include; *Streptococcus species, Escherichia coli, Candida albicans, Klebsiella* species, and Pseudomonas species.

Sensitivity of the Plant Extracts

This was carried out using agar diffusion method and ditch method.

RESULTS

The results of this study are presented in tables 4.1 and 4.2. The results on agar diffusion method (Table 4.1) showed that the all the organisms isolated were resistant to moringa neat and distilled water extracts. All the organisms are sensitive to ethanolic extract of moringa.

Also all the organisms were also resistant to usira neat and distilled water extract. However, *Candida albicans* is sensitive to ethanolic extract of usira while others are not senstitive.

Furthermore, none of the organisms were sensitive to African biters in all the dilutions. Table 4.2 presents the results on ditch method. The results showed that *E. coli*, *Candida albicans*, strep were sensitive while *Pseudomonas species* and *Klebsiella species* are not sensitive.

Table-4.1: Results for Agar Diffusion Method

Extracts	***Pseudomonas***	***E. coli***	***Candida***	***Kleb***	***Strep***
Moringa (neat)	R	R	R	R	-
Moringa (ethanol)	S	S	S	S	-
Moringa (d/water)	R	R	R	R	-
Usira (neat)	R	R	R	R	-
Usira (ethanol)	R	R	S	R	-
Usira (d/water)	R	R	R	R	
A bitters (neat)	R	R	R	R	-
A bitters (dil1)	R	R	R	R	-
A bitters (dil 2)	R	R	R	R	-
A bitters (dil 3)	R	R	R	R	-
A bitters (dil 4)	R	R	R	R	-

Key

R= resistant

S=sensitive

A.bitter= African Bitters; dil; dilution; d/water – distilled water

Table-4.2: Results for Ditch Method

Micro-organisms	**Reaction**
Escherichia coli	Sensitive
Pseudomonas species	Resistant
Candida albicans	Sensitive
Streptococcus species	Sensitive
Klebsiella species	Resistant
Control organism 1	Resistant
Control organism 2	Sensitive

DISCUSSION

Medicinal plants remain feasible source of new compound for the drug development process. Medicinal plants are becoming a very useful economic resource both in agriculture, phytomedicine development and development of new lead compounds (Saetae and Suntornsuk, 2010; Mkoma and Mabiki, 2012). Many plants or plant parts have been studied for their antimicrobial activity (Ahmad *et al*., 1998; Faria *et al*., 2006). In the present investigation extract of the leaves of *Moringa*, Usira and African bitters was screened for its antimicrobial activity.

The present study was conducted to obtain the antibacterial activity of *Moringa oleifera*, *Usira* and *African bitters*. The results showed that ethanolic extract of moringa was sensitive to *Pseudomonas species*, *E. coli*, *Candida* and *Klebsiella* species. This is in line with the study of Mashiar *et al*., (2009) and Kalpana *et al*., (2013). The disc diffusion method was applied to be used in this study. The ethanolic extract has greater antibacterial activity than the neat and distilled water extract. This result is interesting because in the traditional method of treating a bacterial infection, decoction of the plant parts or boiling the plant in water is employed whereas, according to present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results obtained by Nair *et al*., (2005). In a recent study, aqueous extracts of *Moringa oleifera* was found to be inhibitory against many pathogenic bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* in dose dependent manner (Saadabi and Abu, 2011). *Moringa oleifera* extracts was also found to be

inhibitory against *Mycobacterium phlei* and *Bacillius subtilis* (Eilert *et al.*, 1981). Aside from antibacterial activity of *Moringa oleifera*, it also possesses anti-fungal activity (Chuang *et al.*, 2007). Contrary to resistance against *P. aeruginosa* and Candida albicans for *Moringa oleifera* in other studies, one study using ethanolic extract of leaves, seeds and flowers showed the antimicrobial activity against *E. coli, K. pneumoniae*, *Enterobacter* species, *Proteus mirabilis*, *P. aeruginosa*, *Salmonella typhi* A, *S. aureus*, *Streptococcus* and *Candida albicans* (Nepolean *et al.*, 2009).

Gram-negative bacteria have been found to be less susceptible to plant extracts in earlier studies done by other researchers (Kuhnt *et al*., 1994; Afolayan and Meyer, 1995). These consequences suggest that *M. oleifera* leaves used contain bio-components whose antibacterial potentials are highly comparable with that of the antibiotics against all Gram-negative and Gram-positive bacteria tested. The activity of the plant against both Gram negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds in the plant (Siddhuraju and Becker, 2003; Vaghasiya and Chanda, 2007).

The results of this study showed that ethanolic extract of usira leaves were sensitive to candida species. This is in line with the study of Devprakash *et al*., (2011) who reported that ethanolic extract of usira leaves were sensitive to *Escherichia coli, Staphylococcus aureus, Bacilus subtilis* and *Pseudomonas aureginosa.* However, the differences between the sensitivity of this study and that of Devprakash *et al*., (2011) could be due to the methods employed. Disc agar diffusion method and ditch method were employed in this study as against the cylinder-plate (or cup-plate) method and the turbid metric (or tube assay) method used in previous studies. There are two possibilities that may account for the higher antibacterial activity of ethanolic extract are the nature and quantity of active constituents (alkaloids, flavonoids, essential oil, terpenoids, tannins, etc.) and the other is the capacity of ethanol may have yielded a great number of active constituents responsible for antibacterial activity (Kannan *et al*., 2009). Ethanolic extract of *Vetiveria zizanioides* is known to posses flavonoids, alkaloids, terpenoids, saponins, tannins and phenols which either individually or combination exert antimicrobial activity (Devprakash *et al*., 2011). Some study showed that ethanolic extract of *Vetiveria zizanioides* inhibited gram negative bacteria than gram positive bacteria (Devprakash *et al*., 2011).

Furthermore, the result of this study also showed that the all the dilutions of African bitters and usira were not sensitive to the micro-organisms used in this study. This could be possibly be as a result of the method of extraction used in previous studies that reported a higher susceptibility to these organisms. There is no much information on the antibacterial activities of herbal bitters. However, according to Molla *et al*., (2010) who studied antibacterial activity in the leaves of seven bitter medicinal plants of bangladesh reported that these plants were sensitive to *Staphylococcus aureus, Bacilus subtilis, Escherichia coli, shigella species* and *Salmonella typhi*-A.

The possible reason for the non sensitivity of Herbal bitters to the micro-organisms could be attributed to the fact that it works effectively when taken orally because of metabolites in the body. Also the method of extraction and dilutions used in this study could have reduced the potency and concentration of the extracts thereby leading to low susceptibility. Similarly, Mashiar *et al*., (2009) and Kalpana *et al*., (2013) reported they used different solvents such as cold aqueous extracts, hot aqueous extracts, 95% ethanol, 90% chloroform, 80% and petroleum ether. They also reported higher zones of inhibition in their study which could be due to the increased concentration of their extracts as opposed to this study.

The results of this study also showed that the ditch method were more sensitive when compared with the disc diffusion method. However, this variation in sensitivity could be as a result of concentration differences in both methods, with the ditch method having higher concentration of the extracts. Therefore it therefore means that the sensitivity of the extracts to micro-organisms were dose (concentration dependent). Similar observation was made by Devprakash *et al*., (2011); who reported that that increase in concentration of *Vetiveria zizanioides* inhibited gram negative bacteria than gram positive bacteria.

Today, most pathogenic organisms are becoming resistant to antibiotics (Chandarana *et al*., 2005). Moringa leaves have been reported to be a good source of natural antioxidants such as ascorbic acid, avo-noids, phenolics and carotenoids (Dillard and German, 2000). To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Thus, *M. oleifera* and other herbal plants such as Usira and African bitters could become promising natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity will always need to be considered.

From the studies and results obtained, it can be concluded that the extracts of *Moringa* and Usira (ethanol extract) inhibited the growth of bacteria isolates while. Hence, it can be concluded that the leaf of *Moringa* and Usira can be a very good source for herbal drugs especially in using ethanol as a solvents. Since the test isolates show various degree of susceptibility to the extracts, the laboratory should ensure continuous routine sensitivity test for each isolates

and identification of these organisms to assert their antimicrobials sensitivity to *Moringa* and Usira thereby saving cost of treatment of various infections. Also, *Moringa* and Usira should not only be limited to microbiology, further work should be carried out to determine its efficacy in managing other diseases. Other methods of extraction should be employed in extracting medicinal plants active components and if purified, the activities of the extracts can be stronger than the convectional antibiotics.

CONFLICT OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

FUNDING

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHORS' CONTRIBUTIONS

All authors participated in the entire research process.

ACKNOWLEDGEMENTS

The authors would like to thank all the Laboratory and technical staffs of the department of Medical Laboratory Science, Ambrose Alli University Ekpoma, Edo State for their excellent assistance and St Kenny Research Consult, Ekpoma, Edo State for providing medical writing support/editorial support in accordance with Good Publication Practice (GPP3) guidelines.

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