European Journal of Food Science and Technology
Vol.7, No.1, pp.36-43, February 2019
Published by European Centre for Research Training and Development UK (www.eajournals.org)

ANTI-BACTERIAL EFFECT OF COLD WATER EXTRACT OF BITTER LEAF (*VERNONIA AMYGDALINA*) ON SOME SELECTED MICRO ORGANISMS

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ABSTRACT: *Anti-bacterial activities of the leaf of V.amygdalina (bitter leaf) was tested on some microorganisms. Five concentrations 0.5g/ml, 1.0g/ml, 1.5g/m, 2.0g/ml and 2.5g/ml were used and the control experiment was carried out to compare the diameter zones or clearing from the extracts and already standardized antibiotics. Agar well plug method was used for the tests. The bitter leaf extract was made with cold water. Nutrient agar were prepared and inoculated with the different bacteria strains after which wells were made in the in the media and bitter leaf extracts were poured on them. The cold water extracts of V.amygdalina showed inhibitions on the five organisms according to concentration. The organism susceptibility varied with more inhibition to E.Coli, P.aeruginosa, Salmonella typhi, Klebsiella pneumoniae and least to Styaphalococcus aureus.*

KEYWORDS:-Antibacterial, Cold Water Extract, Bitter Leaf, Micro Organisms

INTRODUCTION

There have been a great shift from prescription of antibiotics to the use of medicinal plants. It is estimated there are 250,000 to 500,000 species of plants on earth, and relatively a small percentage of these are used as food by both humans and other species of animals. Frankincense and myrrh probably enjoyed their status of great worth due to their medicinal properties (Mar tinko, 2005).

For years back antibiotics have been critical in the fight against infectious diseases caused by bacteria and other microbes (Cowan 1999).

However disease causing microbes have become resistant to antibiotics drug therapy. Today the effect is less optimistic because many bacterial strains have developed resistance to convectional treatment. The resources allocated to studying the natural plant is only a fraction of those dedicated to developing conventional pharmaceuticals (Allen, 1979).

A natural product is a chemical compound or substance produced by a living organism found in nature that usually has a pharmaceutical drug discovery and drug design (Richard, 1997).

Medicinal herbs are simple native herbs and spices that produce some of the world's best healing agents. Herbal treatments are also used for treating high blood sugar and diabetes, example the plant called Tutsi (Richard 1997).

Vernonia sp. is a genus of about 100species in the family of Asteraceae. Some are known as iron weed, some edible and of economic value. They are known for having intense purple flowers. This genus is named after the English botanist William Vernon. The different species includes *V.calvoana V.amygdalina* and *V.colorata*, and are common in West African and Central African countries (Anonymous, 2000).

ISSN 2056-5798(Print), ISSN 2056-5801(online)

V.amygdalina is well known as medicinal plant with several uses attributed to it including treatment of diabetes, fever, but this can be reduced by boiling or in the young leaves, by soaking in several changes of water (Jones, 2001).

Staphylococcus aereus is gram positive organism with spherical cells usually arranged in grape like irregular clusters. They grow readily in different types or media and produce pigments that vary from white to deep yellow. Pathogenically, it causes boils, pustules, mastitis, septicemia, meningitis and pneumonia (Cheese brough, 2000).

Escherichia coli belong to the large group of gram-ve rods reffered to as entero bacteria. They are naturally found in the intestinal tract, in soil and in water. It is non-sporing and many strains develop capsules and produce a mucoid type of growth in solid media. Pathogenically, *E. coli* causes urinary tract infections, infections of wounds sepsis, diarrhea, infantile disease, desentry and haemorrhageal diarrhea (Cheese brough, 2000).

Klebsiella pneumoniae is a gram-ve, non-motile, capsulated rods. There are four sub-species of klebsiella *Viz-K.preumoniae K.aerogenes*, *K.ozaenae*, and *K.rhinoscleromatis*. Pathrogenically, *K.pneumoniae* causes chest infections and occationally severe bronchiopneumonia with long abscesses (Cheese brough, 2000).

Pseudomonas aeruginosa is a gram-ve, non-sporing motile rod and some strains are capsulated. Commom in hospital environments and therefore nochosomial in nature. It is an obligatory aerobe, usually recognized by the pigments it produces including pyocyannin, a blue-green pigment and pyonverdin a yellow-green florescent pigment.

Salmonella typhi is also gram-ve rods and non-spore forming and non-capsulated. They are aerobic and facultative aerobes. They ferment carbohydrates with acid and gas production.

Pathogenically. It causes the following diseases, enteric fever (typhoid and paratyphoid) with bacteria caused by *S.typhi* and S. paratyphoid A,B,C. These are usually found only in human beings excreta in the faeces and urine of infected patients and carriers (Cheese brough 2000).

The aim of this work was to investigate the anti bacterial effect of V. amygdalina with a view of its possible use as a bacterial control in our food products.

MATERIALS AND METHODS.

Samples were collected from a local market and carried in clean polythene bag for identification in the lab.

PREPARATION OF NUTRIENT AGAR was prepared by dissolving 79 the powder in 250ml of distilled water and heated on a hot plate. The resulting mixture was then sterilized by autoclaving at 121^{o}C for 15minutes.

Preparation of bitter leaf extract was carried out, first by air drying the leaves, pulverising and the weighing of the powder into the following quantities 5g, 10g, 15g, 20g, 25g. To each of these quantities for 24 hours and was then filtered. The filtration was done with sterile filters and then stored in a refrigeration at 40^{o}C until use.

ISSN 2056-5798(Print), ISSN 2056-5801(online)

The viability tests for each isolate were carried out by resuscitating the organism in nutrient agar. Confirmation tests were carried out on the test organisms. E. coli appeared pink to red in color on Mac conkey agar. *S.typhi* appeared black on Mac conkey agar. *Klebsiella sp* also appeared pink to red in color on Mac conkey agar. *S.aureus* appeared yellow in color on mannitol salt sugar. Anti-microbial sensitivity test was carried out using the method of (Calixto 2000).

RESULTS

Results showed the anti-bacterial activities of the cold water extract of the bitter leaf *V.amygdalina* on the five organisms. *E.Coli* exhibited the highest inhibition followed by *P.aeruginosa*, then by *S.typhi* followed by *K.pneumoniae* e and *S. aureus*, that showed the least. The 2.5 glml showed the highest anti bacterial effect on the test organisms.

PLATE 1

Shows the zone of inhibitory of *S.typhi* the inhibitory reaction of the organisms was only on 1.5glml, 2.0glml and 2.5glml, which ranged from 5mm. 8mm and 10mm respectively. The 0.5gml and 1.0glml did not exhibit any inhibitory effect.

For the control experiment, ciproxin showed the highest sensitivity against *S.typhi*, followed by Gentomicine, then by Erethro-mycin, Amoxycillin, Nitro furantin in that order. Naladixic acid, however did not show any effect.

PLATE 2

Showed the zone inhibition of *S.aureus* on the concentration of 2.5glml only with diameter of 10mm. The other concentrations did not show any inhibition.

For the control experiment, erythromycin. gentamicin, naladixic acid and ciproxin were highly sensitive to *S.aureus* while amoxycilin, nitro furantin showed no sensitivity to *S.aureus*.

PLATE 3

Shows the anti-bacterial activities of *V.amygdalina* on *P.aureginosa*, where the 2.5glml concentration gave the widest zone of inhibition. This was followed by 2.0glml, 1.5glml with zones of inhibition ranging from 14mm,10mm, 8mm in diameter respectively, while the zone of 0.5glml and 1.0glml showed no inhibition.

For the control experiment, erythromycin, nitrofurantin and ciproxin were highly sensitive against *P.aureginosa*. Amoxicillin and naladixic showed no inhibition.

PLATE 4

Showed the zone of inhibition of *K.pneumoniae* on the concentration of 2.5glml only which had a diameter of 18mm. The other concentration showed no inhibition.

For the control experiment, ciproxin had the widest zone of inhibition against *K.pneumoniae* on the concentration of 2.5glml only which had a diameter of 18mm. The other concentration showed no inhibition.

ISSN 2056-5798(Print), ISSN 2056-5801(online)

For the control experiment, ciproxin had the widest zone of inhibition against *K.pneumoniae*, followed by Gentamicim and Nitrofurantin. These were followed by Erythroinycin, Amoxicillin and Naladixic acid that showed no inhibition against this organism.

PLATE 5

Shows the result of zone of inhibition against *E.coli*. The concentration of 2.5glml gave the highest inhibition followed by the 2.0glml and 1.5glml with least zone of inhibition shown by the 1.0glml concentration. The zones of inhibitions ranged from 23mm, 20mm, 12mm and 10mm in diameter respectively. The 0.5glml did not give any zone of inhibition.

For the control experiment, Erythomycin, ciproxin and Gentaimycim were highly sensitive to *E*. coli. This was followed by Nitrofurantin and Naladixic acid which were sensitive to *E.coli*. Amaxycilin showed no inhibition on E. coli.

TABLE 1: Show the antibacterial activities of the cold bitter leaf (Vernonia *amygdalina* on *Escherichia coli*, *Pseudomonas aeruinosa*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Staphylococcus aureus*.

PATHOGEN	**0.5gml**	**1.0gml**	**1.5glml**	**2.0glml**	**2.5glml**
Escherichia coli	-	+	+	+	+
Pseudomonas aeruginosa	-	-	+	+	+
Salmonella typhi	-	-	+	+	+
Klebsiella pneumoniae	-	-	-	-	+
Staphylococcus aureus	-	-	-	-	+

+ = Inhibition **- = No Inhibition**

This table shows the effect of cold extract of *V.amygdalina* at the different concentrations on the test organisms. Its effects shows inhibition on *E. coli* at all concentrations except at 0.5glml.On *P.aureginosa* and *S.typhi* inhibitions. While on *K.pneumoniae* and *S.aureus* inhibitions were shown only at 2.5glml concentration.

TABLE 2: Show the zone of inhibition of the five organisms in (mm),the use of six antibiotics.

CONCENTRATION OF COLD WATER EXTRACT OF BITTER LEAF

ANTIBIOTIC DISC	**Escherichia coli**	**Pseudomonas aeruginosa**	**Salmonella typi**	**Klebisella Pneumonia**	**Staphylococcus aureus**
ERYTHROMYCIN	++	++	-	-	++
GENTAMINIC	++	+	+	+	++
AMOXYCILLIN	-	-	-	-	-
NITROFURANTIN	+	++	-	-	-
NALADIXIC ACID	+	-	-	-	++
CIPINROXC	++	++	++	++	++

ISSN 2056-5798(Print), ISSN 2056-5801(online)

Shows the inhibitory effect of the six anti-biotic on the test organisms. The anti-biotic on the test organisms. The anti-biotic erythromycin showed a high inhibitory effect on *E.coli*, *P.aureginosa* and *S. aureus,* but no effect on *S. typhi* and *K.pneumonia*, the organisms *E.coli* and *S.aureus* both showed very high sensitivity to Gentamicin moderately followed by *P.aureginosa*, *S.typhi* and *K.pneumoniae*. Amoxycillin did not have any inhibitory effect on all test organisms. Nitrofuran had a moderate effect on *E.coli*, high effect on *P.aureginosa*, but no effect on *S.typhi*, K.*pneumoniae* and *S.aureus*. Naladixic acid had moderate inhibitory effect on *E.coli*, high effect on *S.aureus* and no effect on *P.aureginosa*, *S.typhi* and *K.pneumoniae*. Ciproxcin was highly effective (highly sensitive)to all the test organism.

DISCUSSION

Results obtained indicated that the bitter leaf extract exhibited considerable anti-bacterial activity against the five species of bacteria used. *E.coli* showed the highest level of susceptibility followed by *P.aeruginosa*, *S. typhi* then by K.*pneumoniae*, with *S. aureus* showing the least inhibition. This is probably due to *E. coli* easily losing its cellular contents once its cell wall is punctured as asserted by Nester et al (2004).

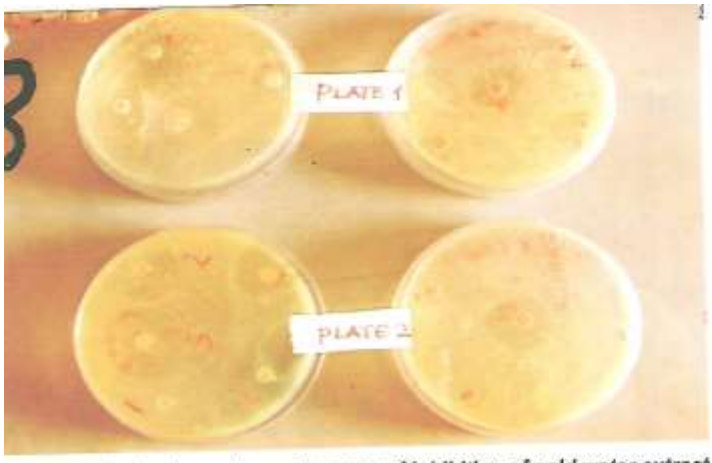
Water extracts of different plants have been used and have shows antibacterial and antifungal activities, that gave significant results. The result obtained have is similar to those of who used water extracts of *A. discoridis*.

The resistance shown by *K. pneumoniae* and *Staphylococcus aureus* (except 2.5gl/ml] is similar to Branter et al 1996;Nostro et al 2000;Ojala et al 2000,who reported that gram-ve bacteria were not susceptible to plant extracts when compared with gram positive bacteria. This resistance of gram negative bacteria is said to be related to lipopolysaccharides in their outer membranes (Gao et al, 1999; Sawer et al 1997). In contrast to this, Foster et al (1990) concluded that gram-positive organisms because of their double cell wall component of mucopeptide and peptidoglycan tends to resist adverse condition better than gram-negative organisms which may be the major cause why *S. aureus* proved to have the least inhibition than the other organisms.

The inhibitory effect of 2.5gl ml concentration on all the test organism shows that high concentrations are desirable for effective control as asserted by Calixto (2000).

Compare with some of the antibiotic discs in the control, *V.amygdalina* has been shown to have very good antibiotic properties comparable to the conclusion of Calixto (2000) which should further be exploited. The results obtained in this work confirms the efficiency of some plant materials as natural anti-microbials and suggest the possibility of using them in drugs for treatment of infectious disease caused by test organisms as also concluded by Obeidat et al (2012).

ISSN 2056-5798(Print), ISSN 2056-5801(online)



Plates 1: of pix. above shows the zone of inhibition of cold water extract of bitter leaf on Salmonella typhi and the control experiment.

Plates 2: of pix. above shows the zone of inhibition of cold water extract of bitter leaf on Staphylococcous-aureus and the control experiment.

Plates 3: of pix. above shows the zone of inhibition of cold water extract of bitter leaf on Pseudomonas aeruginosa and the control experiment.

ISSN 2056-5798(Print), ISSN 2056-5801(online)

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