e-ISSN:2320-3528
p-ISSN:2347-2286

RESEARCH AND REVIEWS: JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Phytochemical Screening and Antimicrobial Activity of Crude Extracts of *Basella alba* and *Helianthus annuus* on Selected Food Pathogens.

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Research Article

Received: 25/01/2014
Revised: 28/02/2014
Accepted: 07/03/2014

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Keywords: Phytochemicals, Antibacterial, Foodborne, Extracts, Inhibition, Diffussion.

ABSTRACT

Phytochemicals composition and antibacterial activity of ethanol and water extract of *Basellaalba* and *Helianthus annuus* were studied against seven selected food borne bacterial pathogens using agar well diffusion and disc diffission methods. The phytochemical analysis revealed the presence of tannin, saponin, alkaloid, terpenoid, flavonoid, glycoside and phenolic compounds at varied composition in both aqueous and ethanol extract of the sample. Quantitatively phytochemical analysis of ethanolic extract of *Basella alba* showed alkaloid to be 3.24%, glycoside 1.34%, Saponin 2.45%, tannin 0.69%, terpenoids 0.04%, flavonoids 1.32% and phenolic compound 0.35% while *Helianthus annuus* showed alkaloid to be 1.23%, glycosides 0.04%, saponin 1.46%, flavonoids 0.03%, terpenoids 0.64% and phenolic compound o.34%.The aqeous and ethanolic extracts showed antibacterial activity against the test organisms.The agar well diffusion showed greater antibacterial activity in *Basellaalba* with better inhibition zones while the disc diffusion method has better activity in *Helianthus annuus* while the ethanol extracts of the plants showed stronger antibacterial activity than the water extracts. The present study re-established the antibacterial activities of the plants extracts which were attributed to the presence of phytochemicals.

INTRODUCTION

The world is fertile with natural and medicinal plants medicinal plants are now more focused than ever because they have the capacity of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacological. The medicinal power of these plants lies in photochemical constituents that cause definite pharmacological on the human body [1]. Photochemical, natural compound occur in plants such as medicinal plants, vegetables and fruits that work with nutrients and fibers to act against diseases or more specifically to protect against diseases [2].The use of plants and plant products medicines could be traced as far as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country [3] . Herbal medicine is still the mainstay of about 75-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades, advances in phytochemistry and in identification of plant compounds, effective against certain diseases have renewed the interest in herbal medicines [4]. The use of plants as source of remedies for the treatment of disease dates back to prehistory and people of all continent have this old tradition. Despite the remarkable progress in synthesis organic chemistry of the twentieth country, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants [5]. Human disease management in Nigeria history also provides evidence of the relationship of plants and medicine [6]. However, research and development on medicinal plants have not advanced to the stage of impacting positively on the health system in Nigeria like other African countries [7] . Plants produce a

e-ISSN:2320-3528
p-ISSN:2347-2286

remarkable diverse array of over 500,000 law molecular mass nature products also known as secondary metabolites. This present study was to investigate the phytochemical constituents and invitro antibacterial activity of extracts of *Basella alba* and *Helianthus annu.*

MATERIALS AND METHODS

Collection of Plant Materials

The fresh leaves of *Basella alba* and *Helianthus annus*used for this work were collected from an uncultivated farm landnear the information village, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria.

Sources of Microorganisms

The bacteria pathogens were those capable of causing food borne diseases. The isolates(*Staphylococcus aureus, Esherichiacoli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella sp, Micrococcus sp and KlebsiellaPneumoniae*) were collected from the Department of Microbiology, Ekiti State University, Ado Ekiti, Nigeria.

Preparation of the Plant Extracts

The leaves of *Basellaalba* and *Helianthus annus*were air dried for 14 days, dry milled into power and packaged into high density polythene to prevent moisture absorption before use. Ethanolic and aqueous extraction was carried out by soaking 50g of powers of *Basellaalba* and *Helianthus annus*separately into 300ml of ethanol and sterile distilled water for 72hours. The extracts were filtered, dried using vacuum rotary evaporator, collected in sterile bottles and stored in refrigerator prior to use.

Antibacterial Activities of the Extracts

The extracts of both plants were tested for their antibacterial properties using the agar well and disc diffusion techniques as described by Alanis [8].

Phytochemical Screening

The quantitative and qualitative phytochemical screening of extracts of the plants were carried out using the modified methods of Trease[9] .Components screened for were glycosides, saponin, flavonoids, tannins, alkaloid, phenolic compounds,terpenoids and steroids.

RESULTS AND DISCUSSION

Table 1: Phytochemical Screening on Basella Alba

Name of compounds	Qualitative test	Quantitative test
Alkaloids	++++	3.24
Glycosides	+++	1.34
Saponin	+++	2.45
Tanin	+++	0.69
Terpenoids	+++	0.04
Steroids	ND	ND
Flavonoids	++	1.32
Phenolic compounds	++	0.35

ND: Not Detected

Table 2: Antimicrobial Activity of Crude Extracts of *Basella alba* by Disc Diffusion and Agar well Diffusion Methods

		Test Isolates/ Zone of Inhibition in (mm)						
Methods	Extracts	SA	KP	PA	BS	EC	SALM	MICRO
Disc diffusion	AQUEOUS	1.1±0.6	1.0±0.9	1.1±0.2	1.1 ±0.4	1.2±0.2	1.1±0.1	1.2±0.2
	ETHANOL	5.7±0.2	6.1±0.2	5.8±0.2	5.9 ±0.2	5.5±0.1	5.7±0.5	6.7± 0.6
Agar well diffusion	AQUEOUS	1.9±0.1	1.29±0.5	2.0 ±0.5	1.58±0.2	1.58±0.2	1.5±0.1	1.55±0.7
	ETHANOL	6.1±0.2	5.8 ±0.6	5.0±20.1	6.1 ±0.1	6.1 ±0.1	5.9±0.9	7.5± 0.2
	GENTAMICIN 10mg/ml	19.0	20.0	18.0	19.0	18.0	21.00	20.00

SA: *Staphylococcus aureus*, KP; *klebsiellapneumoniae*, PA: *Pseudomonas aeruginosa*, BS: *Bacillus subtilis*, EC*: E.coi*, SALM: *Salmonella*sp , MICRO: *Micrococcus*sp

e-ISSN:2320-3528
p-ISSN:2347-2286

Table 3: Phytochemical Screening on *Helianthus annuus*.

Name of compounds	Qualitative test	Quantitative test
Alkaloids	+++	1.23
Glycosides	+++	0.04
Saponin	+++	0.94
Tanin	+++	1.46
Terpenoids	++	0.64
Steroids	Nd	Nd
Flavonoids	+++	0.03
Phenolic compound	++	0.34

ND: Not Detected

Table 4 Antimicrobial activity of Crude Extracts of Helianthus Annuus by Disc Diffusion and Agar Well Diffusion Methods

		Micro-organisms Zone of Inhibition in mm						
Methods	Extracts	Sa	Kp	Pa	Bs	Ec	Salm	Micro
Disc Diffusion	Aqueous	1.1±0.5	1.2±0.1	1.6±0.3	1.7±0.5	1.3± 0.5	1.1± 0.2	1.1± 0.3
	Ethanol	6.1±0.2	5.88±0.7	6.12± 0.3	7.1±0.5	5.5± 0.1	5.6 ±0.2	5.3± 0.2
Agar well	Aqueous	1.9±0.5	1.3± 0.2	1.67± 0.2	2.1±0.1	1.3± 0.1	1.1± 0.5	1.7± 0.1
diffusion	Ethanol	5.8±0.1	5.71±0.2	5.1± 0.5	6.7±0.1	5.8± 0.2	5.2± 0.1	5.5± 0.3
	Gentamicin 10mg/ml	20.0	21.0	19.0	20.0	19.0	22.0	21.0

SA: *Staphylococcus aureus*, KP; *klebsiellapneumoniae*, PA: *Pseudomonas aeruginosa*,
BS: *Bacillus subtilis*, EC*: E.coi*, SALM: *Salmonella*sp , MICRO: *Micrococcus*sp

Table 1 showed the qualitative and quantitative phytochemical analysis of ethanol extracts of *Basellaalba*. The tested bioactive compounds were present. Quantitatively alkaloids was 3.24%, glycoside was 1.34%, saponin was 2.45%, tannin was 0.69%, terpenoids was 0.04%, flavonoids was 1.32%, phenolic compounds was 0.34% and steroids was not detected.Table 3 showed the qualitative and quantitative phytochemical screening of the *Helianthus annuus*ethanol extracts. The compounds screened for were alkaloids 1.23%, glycoside 0.04%, saponin 0.94%, tannin 1.46% terpenoids 0.64%, flavonoids 0.03% and phenolic compounds 0.34% and steroids was not detected.

Table 2 showed the antibacterial activity of ethanol and aqueous extracts of *Basellaalba* on selected food borne bacterial pathogen using disc diffusion and agar well diffusion methods. For the disc diffusion method, the aqueous extract had an inhibition zone of 1.2±0.6 on *Staphylococcus aureus*, 1.0 ±0.9 on *Klebsiella pneumonia*, 1.1± 0.2 on *Pseudomonas aeroginosa*, 1.1±0.4 on *Bacillus subtillis*, 1.2 ±0.2 on *Escherichia coli*, 1.1±0.1 and 1.2±0.2 on *Micrococcus luteus*. The ethanol extract had a higher inhibition zone of 5.7 ±0.2 on *Staphylococcus aureus*, 6.1 ±0.2 on *Klebsiellapneumonia*, 5.8± 0.2 on *Pseudomonas aeruginosa*, 5.9± 0.2 on *Bacillus subtillis*, 5.5 ± 0.1 on *Escherichia coli,* 5.7±0.5 on *Salmonella typharium* and 6.7±0.6 on *Micrococcus luteus.*

For agar well diffusion method, the aqueous extract had inhibition zone of 1.9 ± 0.1on *Staphylococcus aureus*, 1.29 ±07 on *Klebsiella pneumonia*, 2.0 ± 0.5 on *Pseudomonas aeroginosa,* 1.58 ± 02 on *Bacillus subtillus*, 1.58 ± 07 on *Escherichia coli*, 1.5±0.1 on *Salmonella typharium* and 1.55±0.7 on *Micrococcus luteus* while the extracts had 6.1±0.2 on *Staphylococcus aureus*, 5.8± 0.6 on *Klebsiella pneumonia*, 5.02 ±0.1 on *Pseudomonas aeroginosa*, 6.1 ±0.1 on *Bacillus subtilis*, 6.1 ± 0.1 on *Escherichia coli,* 5.9 ±0.9 on *Salmonella typharium* and 7.5± 0.2 on *Micrococcus luteus*.

It could be deduced that the ethanol extract had a better extraction potential than that of distilled water. For the agar well diffusion, it followed the same trend in which the ethanol extract had better antibacterial potential. In both methods, *Staphylococcus aureus* was the most susceptible for both aqueous and ethanol extract.Table 4 showed the antibacterial activity of both ethanol and aqueous extracts of *Helianthus annuus* on selected food borne pathogen using both disc diffusion and the agar well diffusion methods. In the disc diffusion method, the aqueous extract had an inhibition zone of 1.1±0.5 on *Staphylococcus aureus*, 1.2±0.1 on *Klebsiella pneumonia*, 1.6±0.3 on *Pseudomonas aeroginosa*, 1.7±0.5 on *Bacillus subtillis*, 1.3±0.5 on *Escherichia coli*, 1.1±0.2 on *Salmonella typharium*, and 1.1±0.3 on *Micrococcus luteus* while the ethanol extract had 6.1± 0.2 on *Staphylococcus aureus,* 5.88±0.7 on *Klebsiella pneumonia*, 6.12±0.3 on *Pseudomonasaeroginosa,* 7.1 ±0.5 on *Bacillus subtilis*, 5.5 ± 0.1 on *Escherichia coli*, 5.6 ±0.2 on *Salmonella typharium* and 5.3 ±0.2 on *Micrococcus luteus*. For the agar well diffusion method, the aqueous extract had inhibition zone of 1.9± 0.5 on *Staphylococcus aureus*, 1.3 ±0.2 on *Klebsiella pneumonia*, 1.67 ±0.2 on *Pseudomonas aeroginosa*, 2.1 ± 0.1 on *Bacilus subtilis*,1.3±0.1 on *Escerichia coli*, 1.1±0.5 on *Salmonella typharium* and 1.7±0.1 on *Micrococcus luteus*. The ethanol extract had 5.8 ± 0.1 on *Staphylococcusaureus*, 5.71 ±0.5 on *Pseudomonas aeroginosa*, and 5.7 ± 0.1 on *Bacillussubtilis,* 5.8±0.2 on *Escherichia coli*. 5.2 ±0.1 on *Salmonella typharium* and 5.5±0.3 on *Micrococcus luteus*.

The antibacterialactivities of the extracts of the two plants followed the same trend in which the agar well diffusion of the ethanol extract showed better antibacterial potentials. This could be attributed to the direct contact the extracts had with the organisms in the well diffusion and the better extraction of the ethanol over water [10] .Phytochemical screening showed the presence of active pharmacological components such as tannins, saponin, glycoside, flavonoids, alkaloids and phenolic. These components are known to be biologically active because they protect the plant against infections and predations by animals. Phytochemicals generally extends their antimicrobial activities through different mechanisms to that of synthetic drugs [7] .Medically, this is important for the treatment of cancer, gonorrhea, skin diseases, reduced hypocyte number and dyslipidemia. It also plays important roles in herbs for treating dysentery. This justified the use of *Basellaalba* and *Helianthus annuus* in medicine. The medicinal properties of various plant materials and extracts have been recognized and associated with phytochemical antimicrobial properties of substances are desirable tools in the control of undesirable organisms especially in the treatment of infectious disease and in food spoilage.

The active ingredients in plants known as phytochemicals usually interfere with growth and metabolism of microorganism in a negative manner [4] . These phytochemicals like phenolic compounds (tannin) present in the extract of the plant are potent inhibition of microbial growth, saponnin also have great antimicrobial properties [11] . The presence of bioactive substance has been reported to confer resistance to plants against bacteria, fungi and pests and therefore explains the demonstration of antibacterial activities by the plants extracts [4] .[6]reported that the presence of some secondary metabolites in the leaves and roots of some plants inhibited some microorganisms isolated with sexually transmitted infections. Phytochemicals are known to be biologically active and therefore maid the antibacterial properties. These secondary metabolites exert antimicrobial property through different mechanism. Tannins have been found to form irreversible complexes with praline rich protein resulting in the inhibition of protein synthesis. [6]reported that tannins are known to react with proteins to provide the typical tannin effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [12] . These observations therefore support the use of *Basellaalba* and *Helianthus annuus* in herbal cure remedies. [13]reviewed the biological activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that *Basella Alba* and *Helianthus annulus* has potential as a source of important bioactive molecules for the treatment and preventing of cancer. The presence of tannins in *Basellaalba* and *Helianthus annuus* supports the traditional and medicinal use of this plant in the treatment of different ailments. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the eliminations and reduction of human cancer cell lines [7] .Saponin was found to be present in *Basellaalba and Helianthus annuus* extracts and has supported the usefulness of this plant in managing inflammation.

The antibacterial assay was performed using both disc diffusion and agar well diffusion methods and studied which had better cleared zones of inhibition. Table 2 and 4 showed the varied susceptibility of the bacteria isolatesagainst the crude extracts on the basis of zones of growth inhibitions and extracting length of zones of growth of inhibition from different studies vary from one organism to another plants and concentration different [4] . The organisms which are sensitive trend to move away from the region around the extract while those that are resistant show no zones of growth of inhibition. Both the extracts demonstrated antimicrobial activity but higher in ethanol extract in agar well diffusion method[14] .Since activities were seen in both the ethanol and water extracts it means that the crude extract can further be refined into pure form and use it against pathogens that cause infections in local communities.

CONCLUSION

Basellaalba and *Helianthus annuus*possess various pharmacological activities as shown in this present work. However, the presence of bioactive chemicals in *Basellaalba* and *Helianthus annuus*extracts and the ability of the extracts to possess strong antibacterial effect on the selected foodborne pathogens proved them medicinal.

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