Original Research Article **Allied science**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

EVALUATION OF ANTIBACTERIAL ACTIVITY OF PHYTO- NUTRIENTS EXTRACTED IN AQUEOUS AND ETHANOL SOLVENTS FROM *HORDEUM VULGARE, BASELLA ALBA* AND *GUIZOTIA ABYSSINI*

***ZAKIYA TANVEER[1], CH.VENKATA RAMANA DEVI[2], ZUBAIDA AZEEM[3] AND CH. MOHAN[4].**

[1]Shadan institute of P.G Studies,Khairtabad,Hyderabad.
[2]Department of Biochemistry, University college of science,Osmania University, Hyderabad.
[3]Department of Food and Nutrition, University college for women,Koti, Hyderabad.
[4]Biotechnology and Molecular Genetics Laboratory, Department of Botany, Osmania University, Hyderabad-500007,India.

ABSTRACT

Since times, plant food products and plant derived products had been used in daily life for cures and relief from various diseases. Recently, there is a scientific curiosity and certain popularity with regard to screening of essential nutrients from different plant extracts for their pharmaceutical usage.Present study reveals the difference in the antimicrobial activity pattern of extracts from *Hordeum vulgare, Basella alba* and *Guizotia abyssini* in ethanol and aqueous solvents against the pathogenic organisms *E.coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Proteus vulgaris* (ATCC 6380). Among all three plant extracts, *Basella alba* found to shown greater activity with ethanol extract against *E.coli* with a zone of inhibition 23mm with IC50 value 62.64mg/ml, least activity was shown against *Pseudomonas aeruginosa* with a zone of inhibition 7mm with IC50 value 79.29mg/ml, The results of crude extracts were compared with standard antibiotic Ciprofloxacin(100 mcg).

KEYWORDS: **Phyto-nutrients, aqueous extract, ethanol extract, *Hordeum vulgare, Basella alba, Guizotia abyssini,* antibacterial activity.**

***ZAKIYA TANVEER**
Shadan institute of P.G Studies,Khairtabad,Hyderabad.

Received on : 14-08-2016
Revised and Accepted on 14-11-2016
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.1.b152-156

INTRODUCTION

Currently, in india, the use of antibiotics and other growth promoters in farm animals and agriculture is following an increasing trend. This poses a major problem, because this promotes the survival of antibiotic-resistant bacterial strains[1]. Furthermore, once antibiotic-resistant bacteria arises, they tend to spread rapidly. The spread of bacterial resistance to antibiotics takes place by the transmission of related genes which have become highly mobile since antibiotic chemotherapy started. The most disturbing issue is the use of antibiotics in agriculture as growth promoters and for treatment of diseases in intensively reared farm animals. Such use has led to pathogenic bacteria possessing resistance genes and the spread of these genes to the soil bacterial community [2]. Such genes have been characterized as diverse, mobile, and abundant in, for example, Chinese swine farms, where antibiotics and heavy metals used as feed supplements are elevated in manures, suggesting the potential for selection of resistance traits [3]. Also, the unmonitored use of antibiotics and metals is causing the emergence and release of antibiotic resistance genes to the environment. The plant-food byproducts, such as tannins, terpenoids, alkaloids and phenolics found to possess antibacterial activity. Studies on the antimicrobial properties of food by-products have examined the antimicrobial effect of different extracts and have characterized the profiles of active compounds. Avocado peels constitute a by-product that contains different phenolics, including phenolic acids, such as hydroxycinnamic and hydroxybenzoic acids, and several flavonoids found to active against Gram-positive and Gram negative bacteria, E. coli being the most sensitive species[5]. Bergamota peel yields essential oil (*Citrus aurantium bergamia* peel oil) and a by-product resulting from the extraction, which has been characterized as an enriched source of flavonoids, namely eriodictyol, hesperetin, naringenin, and others, which have been found to be active against Gram-bacteria[6,7] . Cacao bean husk, a by-product from cacao processing, contains alkaloids (caffeine, theobromine, and theophylline) and polyphenols, extracts from this by-product have been tested against pathogenic bacteria, with promising results[8]. Coconut palm fronds and the husks of the coconut fruits are extensively used as sources for fibres, which are used for a variety of applications[9]. This raw material has been extracted in water infusion to produce phenolics, in which flavonoids such as catechins and procyanidins have been found, both active against *S. aureus*[10]. The present study had been designed to evaluate antibacterial activity from the aqueous and ethanol extract of *Hordeum vulgare, Basella alba* and *Guizotia abyssini*, were tested against bacterial pathogens namely *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus vulgaris*.

MATERIALS AND METHODS

Collection of plant food products

The seed part of *Hordeum vulgare* and *Guizotia abyssini,* leaf part of *Basella alba* had been collected from local markets in Hyderabad.

Preparation of plant food material extracts

All the plants food material were first cleaned using tap water in order to remove any dirt or debris, and later using sterile distilled water. They were dried in laminar flow biological safety cabinet. The dried and ground plant food material (1.0 kg) was first defatted with petroleum ether and then successively extracted with aqueous and ethanol solvents using soxhlet apparatus for 12 hours and filtered to yield extract. The extract was then concentrated in rotavapour and finally dried to a constant weight. The extract obtained was stored in a refrigerator at 4°C. The dried extract was used for the evaluation of antibacterial activity.

Bacterial strains

Bacterial strains used for the study was *Staphylococcus aureus* (ATCC 25923), *Esherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* (ATCC 6380) were purchased from Hi-Media laboratories.

Culture medium and inoculum preparation

High sensitivity testing agar (Hi-Media) was used for checking antibacterial activity of crude solvent extracts of *Hordeum vulgare, Basella alba* and *Guizotia abyssini* plant against four pathogenic bacterial strains- *Staphylococcus aureus* (ATCC 259230), *Esherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* (ATCC 6380). The microbial strains were cultured on the slants in the sterilized Laminar Air Flow from the pure culture. These cultured slants were incubated at 37 ^{0}C for bacterial growth for 2–3 days. High sensitivity testing agar was mixed at a concentration of 23.4 g/1000 ml in distilled water and autoclaved at 121 ^{0}C for 15 min. A loop full from pure culture of a bacterial strain was mixed in the 10 ml of Nutrient broth medium. And incubated at 37 ^{0}C overnight. The final concentration of the inoculum for the four bacterial strains used in the experiment was 108 (CFU/mL). For every experiment, freshly prepared sterile nutrient broth (10 mL) was inoculated from the slants and the activated culture was used for streaking onto the agar plates for antimicrobial sensitivity.

Agar well diffusion assay

The antibacterial activity of the crude solvent extracts was determined by Agar well diffusion assay [11]. 2.34 g of high sensitivity testing agar was dissolved in 100 ml of distilled water and autoclaved at 121 ^{0}C for 15 min. Before transferring this medium in sterilized petri plates, it was allowed to cool and then was poured into the petri plates and allowed to solidify. After this, it is inoculated with activated culture using sterile cotton swabs. And the wells are created using sterile agar borer and the wells were filled by adding 100µL of crude solvent extracts and were incubated at 37 ^{0}C for 12–24 h. Three replicates were prepared from each sample. The extracts having antimicrobial activity, inhibit the microbial growth and the clear zones were formed. The zone of inhibition was measured in millimeters. All the analyses were applied in triplicates[12].

Determination of the minimal inhibitory concentration (mic)

The protein extracts of three selected plant food material were tested against the reference strains for

antibacterial activity micro-dilution method in 96 well microliter plates [13] with minor modifications and recommended by the National Committee for Clinical Laboratory Standard [14]. The antimicrobial activity of the extracts was evaluated against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* (ATCC 6380). Briefly, antimicrobial activity was carried out in 96 well microliter plate containing different concentrations of extracts. The culture suspension (100 µL) was added to each well having 105 CFU/mL and final volume was made to 200 µL by adding LB broth. Plates were incubated at 37 ± 1°C for 18 h and then 10 µL of MTT (5 mg/mL) was added to each well. The plates were examined with ELISA reader (TECAN) at 530 nm and the lowest concentration of each protein extract which showed complete inhibition was taken as its minimum inhibitory concentration (MIC). In control experiments, sterile distilled water and ethanol were added in place of plant extracts. Whereas, antibiotics Ciprofloxacin (100mcg) were used as positive controls. For blank reaction, the sterile broth was used in place of suspension cultures (without inoculum).

RESULTS AND DISCUSSION

In the present study the ethanol and aqueous extracts of *Hordeum vulgare, Basella alba* and *Guizotia abyssini* were tested against four pathogenic bacterial strains [*Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* (ATCC 6380)]. The diameter of zone of inhibition and IC50 values were calculated. Among all the three plants ethanol extract of *Basella alba* were found to shown highest diameter of zone of inhibition 23mm and IC50 value 62.64 (mcg/ml) [Table 2][Figure 2], which is found to be more than standard antibiotic ciprofloxacin(100mcg) that had shown only 14mm diameter of zone of inhibition with IC50 value 92.489 (mcg/ml), the same extract was found to shown lowest diameter of zone of inhibition 7mm with IC50 value 79.29 (mcg/ml).The extracts of *Hordeum vulgare* and *Guizotia abyssini* shown moderate antibacterial activity with diameter of zone of inhibition ranges from 10-14mm (Table 1) (Figure 1) and 11-16mm (Table 3) (Figure 3). All the plant food extracts shown good antibacterial activity on selected bacterial strains. In comparisons with our study, similar research is being carried out with different plant food products as crude extracts from potato peel have been tested against Gram-positive and Gram-negative bacterial strains, bacteriocidal and bacteriostatic effects have been noted, but only at high concentrations [15]. Extracts from tamarind stem, bark, and leaves were assayed against Gram-positive and Gram-negative bacteria, fungi, and yeast, phytochemical analyses have yielded phenolics, saponins, alkaloids, and essential oils. The authors indicated that this plant has a broad spectrum of antibacterial activity [16]. Extracts from tomato seeds were found active against Gram-positive bacteria, Enterococcus faecalis proving to be the most susceptible and antifungal activity was found against *Candida albicans* [17].

Table 1

Antibacterial activity of differents solvent extracts from Hordeum vulgare, Diameter of zone of inhibition[mm] and IC50 values[mcg/ml].

S.No	Aqueous		Ethanol		Cipro (100mcg)	
	ZOI	IC50 Value	ZOI	IC50 Value	ZOI	IC 50 Value
S .aureus	13	27.64	12	25.91	16	160.529
E .coli	12	67.56	14	64.12	14	92.489
P.aeruginosa	10	28.01	10	68.33	12	144.634
P.vulgaris	13	59.68	13	57.83	14	72.685

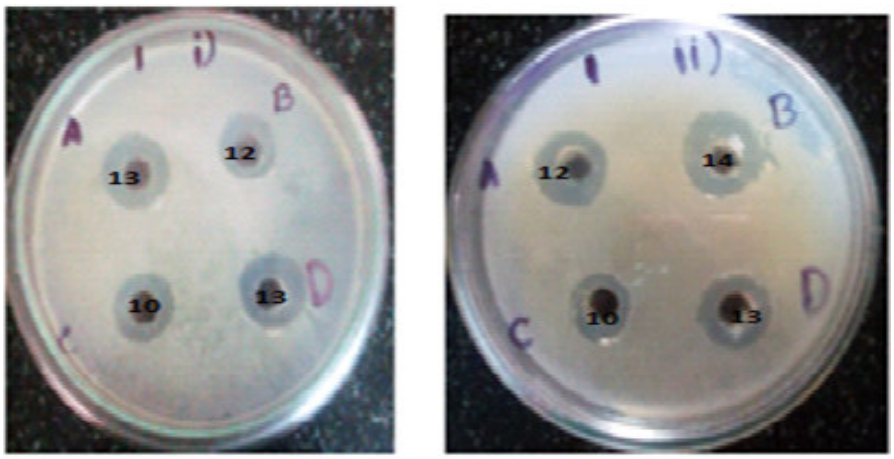

Figure 1

Represents diameter of zone of inhibition of differents solvent extracts from Hordeum vulgare. Staphylococcus aureus [A], Escherichia coli [B], Pseudomonas aeruginosa[C] and Proteus vulgaris[D]. Aqueous[i], Ethanol[ii].

Table 2

Antibacterial activity of differents solvent extracts from Basella alba, Diameter of zone of inhibition[mm] and IC50 values[mcg/ml].

S.No	Aqueous		Ethanol		Cipro (100mcg)	
	ZOI	IC 50value	ZOI	IC 50value	ZOI	IC 50Value
S .aureus	13	29.16	13	19.97	16	160.529
E .coli	16	58.38	23	62.64	14	92.489
P.aeruginosa	11	66.18	7	79.29	12	144.634
P.vulgaris	11	56.84	11	37.34	14	72.685

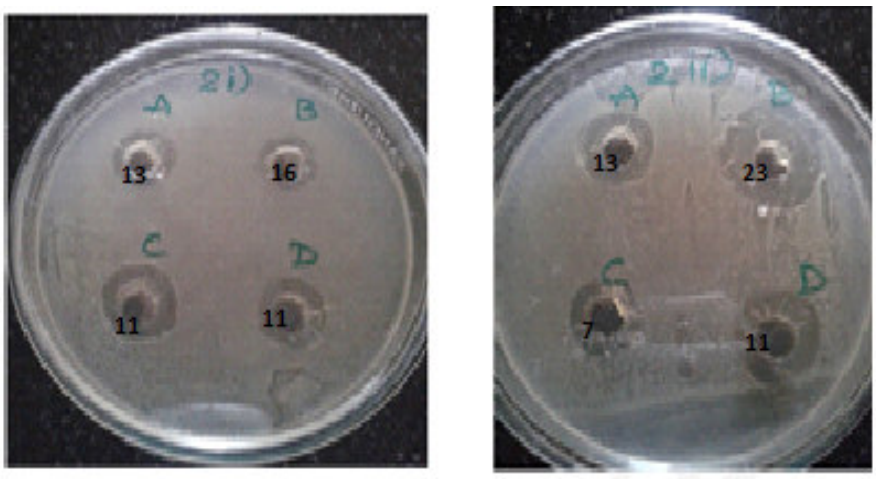

Figure 2

Represents diameter of zone of inhibition of differents solvent extracts from Basella alba. Staphylococcus aureus [A], Escherichia coli [B], Pseudomonas aeruginosa[C] and Proteus vulgaris[D]. Aqueous[i], Ethanol[ii].

Table 3

Antibacterial activity of differents solvent extracts from Guizotia abyssini, Diameter of zone of inhibition[mm] and IC50 values[mcg/ml].

S.No	Aqueous		Ethanol		Cipro(100mcg)	
	ZOI	IC 50value	ZOI	IC 50value	ZOI	IC 50Value
S .aureus	12	47.65	13	54.29	16	160.529
E .coli	13	49.01	14	116.18	14	92.489
P.aeruginosa	11	115.69	16	121.46	12	144.634
P.vulgaris	12	115.69	15	108.65	14	72.685

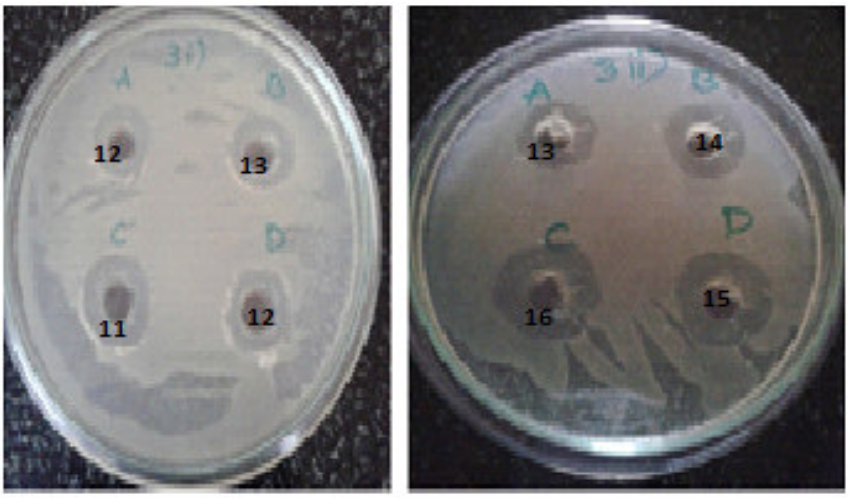

Figure 3

Represents diameter of zone of inhibition of differents solvent extracts from Guizotia abyssini,. Staphylococcus aureus [A], Escherichia coli [B], Pseudomonas aeruginosa[C] and Proteus vulgaris[D]. Aqueous[i], Ethanol[ii].

CONCLUSION

This study supports the usage of plant food products in the form of phyto- nutrient extracts to treat bacterial infections both in animals and human ailments to combat antibiotic resistance and safe environment from bacterial resistance. These finding are key to further screening and purification of phyto- nutrients and their specific antibacterial assessment.

Abbreviation

ATCC-American type culture collection.
CFU = Colony forming unit.
IC50 = Half maximal inhibitory concentration.
MIC= Minimum inhibitory concentration.

ACKNOWLEDGEMENT

We are grateful to the department of Biochemistry, university college of science, Osmania University, Hyderabad, for carrying out work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Okeke IN., Lamikanra A and Edelman R. Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerging Infectious Disease. 5: 18 1999.
2. Seveno NA., Kallifidas D., Smalla K., van Elsas JD., Collard JM., Karagouni AD and Wellington EM. Occurrence and reservoirs of antibiotic resistance genes in the environment. Reviews in Medical Microbiology. 13: 15–27 2002.
3. Zhu YG., Johnson TA., Su JQ., Qiao M., Guo GX., Stedtfeld RD., Hashsham SA and Tiedje JM. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc Natl Acad Sci. USA. 110: 3435–3440 2013.
4. Woappi Y., Gabani P., Singh A and Singh OV. Antibiotrophs: the complexity of antibiotic-subsisting and antibiotic-resistant microorganisms. Crit Rev Microbiol. 1101–1114 2014.
5. Rodriguez-Carpena JG., Morcuende D., Andrade MJ., Kylli P and Estévez M. Avocado (*Persea americana* Mill.) phenolics, in vitro antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. J Agric Food Chem. 59: 5625–5635 2011.
6. Al-Delaimy KS and Ali SH. Antibacterial action of vegetable extracts on the growth of pathogenic bacteria. J Sci Food Agric. 21: 110–112 1970.
7. Mandalari G., Bennett RN., Bisignano G., Trombetta D., Saija A., Faulds CB., Gasson MJ and Narbad A. Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia Risso*) peel, a byproduct of the essential oil industry. J Appl Microbiol. 103: 2056–2064 2007.
8. Cuellar O., Quím T and Guerrero G. Antibacterial activity of the cacao bean husk, *Theobroma cacao* L. Rev MVZ Cordoba. 17:3176–3183 2012.
9. Reddy N and Yang Y. Coconut Husk Fibers, In: Innovative Biofibers from Renewable Resources. Springer Heidelberg, Berlin. 31–34 2015.
10. Esquenazi D, Wigg MD, Miranda MM, Rodrigues HM, Tostes JB., Rozental S., da Silva AJR and Alviano CS. Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera* Linn. (Palmae) husk fiber extract. Res Microbiol. 153: 647–652 2002.
11. Reeves DS. Antibiotic assays. In: Hawkey PM, Lewis DA (Eds.). Medical Bacteriology. A Practical Approach. IRL Press,Oxford. 195–221 1989.
12. Black JG. Microbiology Principles and Explorations. New York. John Wiley and Sons Inc. 5th edn: 336–366 2002.
13. Camporese A., Balick MJ., Arvigo R., Esposito RG., Morsellino N., De SF and Tubaro A. Screening of anti-bacterial activity of medicinal plants from Belize (Central America). J.Ethnopharmacol. 87:103-107 2003.
14. National Committee for Clinical Laboratory Standards. Performance Standards for Anti-Microbial Susceptibility Testing: Eleventh Informational Supplement. NCCLS. Document M100-S11 2001.
15. Sotillo DR, Hadley M and Wolf-Hall C. Potato peel extract a nonmutagenic antioxidant with potential antimicrobial activity. J Food Sci. 63: 907–910 1998.
16. Doughari JH. Antimicrobial activity of *Tamarindus indica* Linn. Trop J Pharm Res. 5: 597–603 2007.
17. Taveira M., Silva LR., Vale-Silva LA., Pinto E., Valenta OP., Ferreres F., Guedes De Pinho P and Andrade PB. *Lycopersicon esculentum* seeds: an industrial byproduct as an antimicrobial agent. J Agric Food Chem. 58: 9529–9536 2010.