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# Appraisal of Antimicrobial Activity of Malted Psedocereals: *Amaranthus cruentus (Amaranth)*And *Fagopyrum csculentum (Buckwheat)*

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## ABSTRACT

The aim of this work was to evaluate antibacterial activity of malted extracts of *Amaranthus cruentus* (Amaranth) and *Fagopyrum esculentum* (Buckwheat); pseudocereals with plain water and alkali 2% $NaHCO_3$against the eight species of Gram-positive (*Staphylococcus auerus, Streptoccocus pyogenes. ,Bacillus cereus)* and Gram-negative bacteria (*Escherichia coli,Proteus vulgaris, Shigella sp., Klebseilla pneumonia, Psedomonas aeruginosa*). Antimicrobial activity of extracts was tested by the disc diffusion method according to the National Committee for Clinical Laboratory Standards Guidelines.It was observed in the present result that *Fagopyrum esculentum and Amaranthus cruentus* alkali (2% $NaHCO_3$) treated extract was found to have maximum zone of inhibition against *E.coli (12.3±0.41mm and 10.5±0.75mm) and B. cereus (10.0±0.2mm and11.1±0.10mm)* while the minimum zone of inhibition was against *streptococcus (5.3±0.30 mm)* for amaranth alkali malted extract.Thus, the findings suggest that malted extract of buckwheat and amaranth may be considered as moderate antibacterial agent against the food borne pathogens and could be used as natural ingredients with their antimicrobial effects in food industry.

**KEYWORDS:** *Amaranthus cruentus, Fagopyrum esculentum,* Antimicrobial activity, Pseudocereals

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## INTRODUCTION

The advancement of bacterial resistance to currently available antibiotics has necessitated the research for novel and effective antimicrobial compounds. It is known that localplants have medicinal properties and this has made traditional medicine cheaper than modern medicine. Globally, plant extracts are employed for their antimicrobial, antifungal and antiviral activities. In particular, the antimicrobial activity of plant extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, and alternative medicine. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm theactivity and to ascertain the parameters associated with it.

Many pathogenic microorganisms, including *Salmonella, Escherichia coli, Fusarium spp. Aspergillus spp. and Rhizopus spp.,* are considered as the contributory agents of food borne disease or food spoilage which are the most vital problem in the food industry[1]. Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against food borne pathogens[2].

In spite of modern improvements in food production techniques, food safety is an increasingly important public health issue[3]. Illnesses caused due to the consumption of foods contaminated with pathogens have a wide economic and public health impact worldwide[4]. Therefore, there is need for new methods of eliminating food borne pathogens, possibly in combination with existingprocessing methods. One such possibility is the use of plant extracts or essential oils as antibacterial additives. Consumer demand for reduced usage of synthetic preservatives has led to research and use of "naturally derived" antimicrobials. In modern food industries mild processes are applied in order to obtain safe products which have a natural or ''green'' image. Under these conditions the antimicrobial effects of plant extracts intend to reduce the proliferation of food borne pathogens[5].

Today a few plant species among all the varieties available for human nutrition are employed commercially on a very large scale wheat, maize and rice are the three cereals dominating the nutrition within the world's population, even if appropriate alternatives are available[6].According to the "International AACC (American Association of Cereal Chemists) has recognized pseudo cereals as grains" and could be a good substitute for cereals in allergic persons, it was decided to compare allergy free pseudocereals: quinoa, buckwheat and amaranth with cereal and legume on the basis of their antioxidant status[7]. In the last years, pseudocereals including amaranth and buckwheat have gained

broad use not only in the common diet but also in the diet of people with celiac disease or allergies to typical cereals[8].The dietary changes required by the celiac patient to begin and maintain a strict gluten free diet are considerable and may have a significant impact on daily life[9].

Buckwheat and Amaranth have high nutritional and functional values as they contain protein with favorable amino acid composition, vitamins, starch, dietary fiber, essential minerals and trace elements[10] and bioactive compound with antioxidant potential[11]. In recent years, the interest about plant extracts has still increase due to their potential as source of natural ingredients with antioxidant, antibacterial or antifungal properties[12] and also considered as potential therapy to treat various chronic diseases, such as diabetes, hypercholesterolemia, hypertension, cardiovascular disorder[13].

Therefore, the aim of the study was to evaluate the antibacterial activity of maltedtreated with plain water and alkali pseudocereals extract.

## MATERIALS AND METHODS

### Collection of PlantMaterial and Preparation of Extract

Pseudocereal;*Fagopyrum esculentum and Amaranthus cruentus*were collected from the Indian Agriculture Research Institute (IARI).Firstly, both the pseudocereals were well washed and removed dirt particles. The seeds were malted with plain water and alkaline solution ($NaHCO_3$) to develop flours, the flour samples of 10 g were immersed in 100ml of distilled water, mixed and allowed to steep for 10 h and germinated for 24 h and kilning wasperformed for 4h then dried to get malted flours.

### Test Microorganisms

Test microorganisms which were used in this experiment are Gram positive (*Staphylococcus auerus* ( MTCC code-7443) , *Streptoccocus* pyogenes (MTCC code- *1924*) , *Bacillus cereus* (MTCC code - 1845) and Gram negative (*Escherichia coli* ( MTCC code- 433), *Proteus vulgaris* (MTCC code - 744), *Shigella sp.* (MTCC code - 1457),, *Klebseilla pneumonia* (MTCC code - 109), *Psedomonas aeruginosa* (MTCC code - 4676) bacteria.

### Culture Medium

The nutrient agar was prepared by dissolving 5 g peptone, 1.5 g beef extract, 1.5 g yeast extract, 5 g NaCl and 20 g agar in 1000 ml distilled water and boiling the solution. The pH was adjusted to 6.4–6.8

and sterilized by autoclaving at 15 psi pressure (121 °C) for 20 min. Sterilized petriplateswere prepared with an equal thickness of nutrient agar. Test bacteria were grown overnight at 37 $^0$C, 120 rpm in 10 ml nutrient broth. This broth was used for seeding the agar plates.

**Antibacterial Assay**

Antimicrobial activity was tested by the disc diffusion method. The 400 µl of the extracts was impregnated onto a small disc of filter paper (diameter 6.0 mm) and placed on top of the seeded medium. Each disc containing 4 mg ml$^{-1}$extract was tested againstgram positive (*Staphylococcus Auerus, Streptococus Bacillus cereus*) and gram negative (*Escherichia coli, Proteus vulgaris, Shigellasps., Klebseilla pneumonia, Psedomonas*).The antibacterial assay plates were incubated at 37 °C for 24 h. The control experiment was carried out to compare the diameter zone of clearing from the extracts and already standardized antibiotics. The antibiotic used was Gentamycin as a standard. The standard discs of the antibiotic Gentamycin (10 µg per disc) served as positive antibacterial control. The diameter of the zones of inhibition around each of the discs (disc diameter included) was taken as measure of the antibacterial activity. The diameters of the zones of inhibition by the samples were then compared with the diameter of the zone of inhibition produced by the standard antibiotic disc used. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded and activity index was calculated by inhibition of the sample divided by inhibition of standard[14].

$$\text{Activity Index (AI)} = \frac{\text{Mean of Zone of Inhibition of the extract (mm)}}{\text{Zone of Inhibition obtained for standard (mm)}}$$ [15]

**RESULTS AND DISCUSSION**

The plant extracts show strong activity if inhibition zone is ≥18 mm, moderate activity if inhibition zone is 13-18 mm, intermediate if inhibition zone is 10-13 mm and no inhibition if inhibition zone is ≤ 10 mm[16]. The Amaranth and Buckwheat maltedextracts (plain water and2% $NaHCO_3$)weretested in 4 mg/ml concentration. The results shown in Table 1 indicate that analyzed extract had different antimicrobial effect depending on applied bacterial strains. Tested malt extracts showed antimicrobial activity on all selected strain by screening with disc diffusion method accept *S. aureus* (positive) and *pseudomonas* (negative) bacteria.

Among all tested extracts, (Buckwheat)2%$NaHCO_3$treated malted extract was found to be most active than corresponding extracts (Table1). Plain watertreated malt extracts of both amaranth and buckwheat

has shown zone of inhibition against six tested gram positive (*Staphylococcus auerus, Streptoccocus Bacillus cereus*) and gram negative bacteria (*Escherichia coli, Proteus vulgaris, Shigellasps.,Klebseilla pneumonia, Psedomonas*). On the other hand, alkali treated maltextract of both flours was efficient against seven out of eight tested bacteria (*Streptococus, Staphylococcus aureus Bacillus cereus Escherichia coli,Proteus vulgaris, Shigella spp. Klebseilla pneumoniae, Psedomonas*). Buckwheat alkali treated malt extract was observed to have maximum zone of inhibition against *Ecoli* (12.4 ±0.41mm) and amaranth alkali maltextract for *Bcereus* (11.1±0.10 mm)while the minimum zone of inhibition was seen in *Strptococcus* (5.3±0.30 mm) for amaranth alkali malted extract. As depicted in figure 1 and 2 that alkali treated malt extracts have shown better activity the tested pathogenic organisms.Similarly, activity index of the flour extracts varies from 0.22 to 0.49. The maximum activity index was 0.49 for Buckwheat alkali malted extract (*E coli*) to the minimum 0.22 for amaranth alkali malted extract (*streptococcus*). No inhibition was observed against *S. aureus* (positive) and *pseudomonas* (negative) bacteria.

There is confirmation given[17] that *Cynodondactylon* were valuable antimicrobial agent for similar pathogenic organism. Amaranth and buckwheat extracts has strong antimicrobial effect particularly against fungi and yeast[18]. Antimicrobial activity could be attributed to the presence of flavonoids in buckwheat[19]. Since flavonoids are known to be synthesized by plants in response to microbial infection[20].it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms.

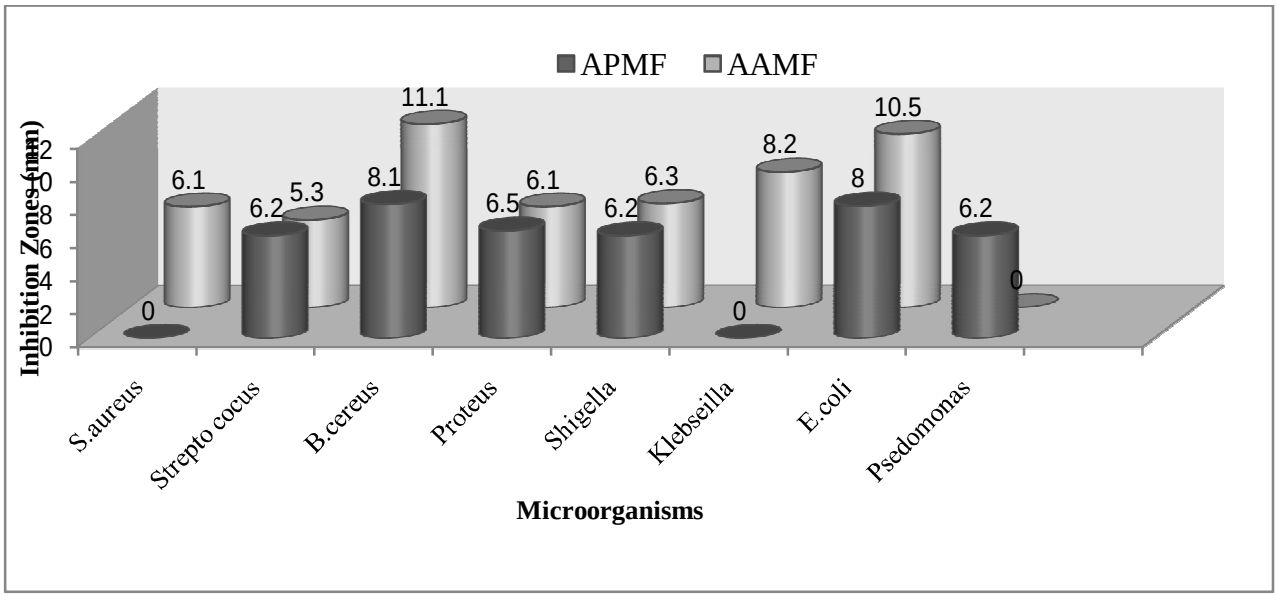


**Figure No. 1: Antimicrobial activity of malted *Amaranthu scruentus* (Amaranth) APMF= Amaranth plain water malted flourAAMF= Amaranth alkali malted flour**

**Table No.1 : Antimicrobial Activity of Malted *Amaranthuscruentus* (Amaranth) and *Fagopyrumesculentum* (Buckwheat) Flours**

| Malted Extracts (4 $mgml^{-1}$) | Inhibition zones (mm) and AI (Activation Index) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Gram positive bacteria | | | Gram negative bacteria | | | | |
| | *S.aureus* | *Streptcocus* | *B.cereus* | *Proteus* | *Shigella* | *Klebseilla* | *E.coli* | *Pseudomonas* |
| **Amaranth (Plain$H_20$)** | NIL | 6.2 ±0.25 | 8.1 ±0.15 | 6.5 ±0.37 | 6.2 ±0.10 | NIL | 8.0 ±0.1 | 6.2 ±0.15 |
| **AI** | **NIL** | **0.25** | **0.32** | **0.28** | **0.25** | **NIL** | **0.32** | **NIL** |
| **Amaranth ($NaHCO_3$)** | 6.1 ±0.15 | 5.3 ±0.30 | 11.1 ±0.10 | 6.1 ±0.05 | 6.3 ±0.02 | 8.2 ±2.6 | 10.5 ±0.75 | NIL |
| **AI** | **0.21** | **0.22** | **0.44** | **0.26** | **0.26** | **0.35** | **0.42** | **NIL** |
| **Buckwheat (Plain $H_20$)** | NIL | 6.4 ±0.36 | 9.1 ±0.1 | 6.7 ±0.15 | 10.2 ±0.10 | 6.1 ±3.57 | 10.3 ±0.41 | NIL |
| **AI** | **NIL** | **0.26** | **0.36** | **0.29** | **0.42** | **0.26** | **0.41** | **NIL** |
| **Buckwheat ($NaHCO_3$)** | NIL | 6.4 ±0.40 | 10.0 ±0.2 | 6.8 ±0.10 | 6.2 ±0.05 | 7.2 ±2.37 | 12.4 ±0.20 | 6.2 ±0.02 |
| **AI** | **NIL** | **0.26** | **0.4** | **0.29** | **0.25** | **0.31** | **0.49** | **0.25** |

**Zone are Mean ±SD for n=3, Nil:No Inhibition .Standard for Antibacterial-Gentamycin**
***S.aureus(28), Streptococus (24),B.cereus( 25), Proteus(23), Shigella (24) ,Klebseilla (23),E.coli(25),Psedomonas(24)***

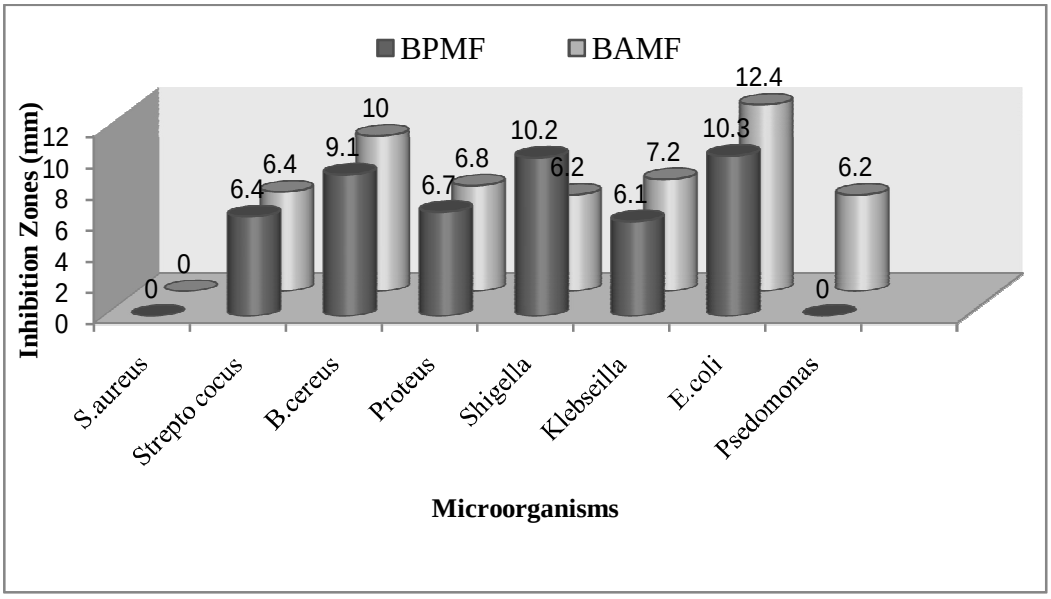


**Figure No.2: Antimicrobial activity of malted *Fagopyrum esculentum* (Buckwheat)**

BAMF= Buckwheat Alkali Malted FlourBPMF= Buckwheat Plain Water Malted Flour

## CONCLUSION

Presented result suggests that alkali ($NaHCO_3$) treated malted buckwheat has more effective against the selected pathogenic organisms than plain water treated malted extract. From this work it can be said that alkali treated malt extract of pseudocereals have effective and potential source of compounds with antimicrobial activity against bacterial strains in food and also it indicates alkali treated malt extract may be an ideal component for possible food preservation by natural plant based products. Thus, the result are encouraging enough to pursue by activity guided fractionation of this extracts and therefore, the use of this pseudocereals by the traditional healers for the treatment of the aforementioned diseases have been validated. Further research is necessary to determinetheir full continuum of efficiency for the antibacterial, antifungal as well as antimycotic activity from the pseudocereals malted flours. Further more different extraction solvents and procedures could be investigated.

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