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In-Vitro Antibacterial Activity of Underutilized Plant Crude Extracts Against FoodBorne Pathogens

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1. Abstract

This study aims to determine the antibacterial activity of underutilized plants "Kottamba" (*Terminalia catappa),* "Purpurata" *(Alpinia purpurata)* and *"*Harankaha*" (Cucurma zedoria)* against six strains of *Staphyloccocus aureus*, four strains of *Listeria monocytogenes*, *Escherichia coli* and *Salmonella Typhimurium*.

Crude rhizome extracts were obtained for all plants while *T. catappa*, red *peri*-carp of the fruit was used. The antibacterial activity was determined using agar disc diffusion and broth dilution assay. Total phenol content and Gas Chromatograph-Mass Spectrometry analysis were performed only with plant which showed the strongest antibacterial active. Among the plant extracts, *T. catappa* extract showed significantly ($p<0.05$) higher DIZ (19.6±0.47 mm) against *S. aureus* 113. *A. purpurata* showed DIZ ranging (16.3±0.54, 15.0 ±1.00, 14.3 ±0.57mm) against *L. monocytogenes V7 (1/2a), S. aureus 25925* and *S. aureus MSSASS 25D* respectively. However, *C. zeodria* showed the significantly ($p<0.05$) lowest DIZ for all tested bacteria strains. The MIC of the *T. catappa* ethanol extract was 10 mg/ml, while MBC was 80 mg/ml for the *S.aureus* 113 strain tested. *T. catappa* (ethanol) exhibited the phenolic content 81.54±1.28 mg GAE/g dry sample and major compound (31.86 %) as 2, 5-Furandione, 3 methyl. These results confirmed that *T. catappa* has a potential antibacterial activity to serve as bio preservative.

Keywords: antibacterial activity, Gas Chromatograph Mass Spectrometry, plant, Total phenol content

2. Introduction and research problem/issue

Food borne disease is a global issue with significant impact on human health. The widespread use of antibiotics to control diseases promotes the spread of antibacterial resistance. There is a need to reduce the overuse of antibiotics and to continue studies to develop new drugs, either synthetic or natural. However, consumers are unwilling to use synthetics drugs due to its side effects (George, 2011). There

is a growing consumer demand for more natural antimicrobials as they are considered safe. But the potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically (Alawa, 2012).Therefore, plants should be thoroughly investigated for a their potential to serve as bio preservatives, safety and efficacy.

Terminalia catappa has been used for the preparation of traditional medicine and antibacterial activity was mostly reported on leaves (Akharaiyi, Ilori, & Adesida, 2011), bark (Nagappa, Thakurdesai, Rao, & Singh, 2003) and roots (Abiodun et al., 2015). However, very few literatures were reported on *T. catappa* fruit extracts. *Alpinia purpurpata* have been investigated antimicrobial activity based on plant tissue was rhizomes, leaves and roots. However we could not find literature on antibacterial activity of hexane extracts of rhizome of the plants for methillin resistance *S. aureus*, *L. monocytogenes*. Also antimicrobial activity of *C. zedoaria* rhizome extract against *L. monocytogenes, S.*Typhemurium *and E.coli* was not investigated.

The objective of this study was to find the effect of antibacterial activity of *T. catappa*, *A. purpurata* and *C. zedoria* against selected food borne pathogens. Further, total phenol content and chemical composition of most active antibacterial extract was determined.

3. Research Methodology

The rhizome of *A. purpurata* and *C.zedoaria* and red peri-carp of fruit *of T. catappa* was used for the hexane and ethanol solvent extraction. The antibacterial activity of the hexane and ethanol extract was determined using food-borne bacteria. *S. aureus* 113, *S. aureus* MSSA SS 25D, *S. aureus* MSSA SS 21D, *Listeria monocytogenes* Scott A (4b), *L. monocytogenes* V7 (1/2a), *L. monocytogenes* EDG, *S. aureus* ATCC 29213, *S. aureus* ATCC 49476, *S. aureus ATCC* 25925, *L. monocytogenes ATCC* 7644, *Escherichia coli* ATCC 1858 (*E. coli*) and *Salmonella* Typhimurium ATCC 14028 (*S.* Typhimurium) were used.

Antibacterial activities of the extracts were evaluated using slightly modified disk diffusion method described previously by (Barry, 1976). Minimum Inhibitory Concentration (MIC) and *Minimum Bactericidal Concentration (*MBC*)*, was

performed to the plant with the strongest antibacterial activity showed in disk diffusion assay using quantitative two fold serial dilutions of extracts against *S. aureus 113* and *S. aureus ATCC29213* with some modifications of the method

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described by (Hennekinne, De Buyser, & Dragacci, 2012). Total phenol content and Gas Chromatograph-Mass Spectrometry analysis were performed to the plant with the strongest antibacterial activity.

4. Results and findings

4.1 Disk diffusion assay method

The mean DIZ of *A. purpurata*, *T. catappa* and *C.zedoaria* measured in disk diffusion assay are shown in the Table 1. There was a significant variation ($P<0.05$) observed antibacterial activity between hexane and ethanol extract for each tested underutilized plant. Compared to other plants tested crude ethanol extract (0.5 mg/ml of 10 µl) of *T. catappa* showed significantly ($p<0.05$) higher DIZ 19.6±0.47, 19.3±0.47 mm against *S. aureus* 113 and, *S. aureus* ATCC29213 respectively. *A. purpurata* extract showed a significantly ($p<0.05$) larger DIZ with *L. monocytogenes V7 (1/2a)* irrespective for the

solvent. *A. purpurata* ethanol extract showed significant($p>0.05$) inhibition (9.3± 0.57 mm) against *E. coli* ATCC 1858. Similarly, *C. zedoaria* showed significant ($p>0.05$) inhibition (8.6± 0.57 mm) against *S.* Typhimrium ATCC 14028.

Table 1. Antibacterial activity (Diameter inhibition zone) of underutilized plant extracts

	A. purpurata		*T. catappa*		*C.*
Micro organism	Hexane	Ethanol	Hexane	Ethanol	Hex
Gram Positives					
S. aureus 25925	15.0±1.00^{cdef}	11.3±0.57^{ijk}	6.0±0.00^{n}	17.6±0.47^{bc}	10.
S. aureus MSSA SS 25D	14.3±0.57^{defg}	9.3±0.57^{op}	6.0±0.00^{n}	19.0±0.81^{ab}	9.0
S. aureus ATCC29213	14.3±0.57^{defg}	10.6±0.57^{klm}	6.0±0.00^{n}	19.3± 0.47^{a}	12.
S. aureus MSSA SS 21D	14.0±0.00^{efgh}	10.3±0.57^{lm}	9.6±0.00^{mno}	11.6±0.57^{hij}	11.
S. aureus ATCC 49476	15.0±1.00^{cdef}	11.3±0.57^{ijk}	6.0±0.00^{n}	18.6±0.94^{ab}	9.3
*S. aureus*113	13.0±1.73^{fgh}	12.3±0.57^{hij}	6.0±0.00^{n}	19.6± 0.47^{a}	11.
L. monocytogenes Scott A	14.0±0.64^{efgh}	13.6±0.57^{fgh}	6.0±0.00^{n}	12.6±0.57^{ghi}	7.1
L. monocytogenes V7 (1/2a)	16.3±0.94^{bcd}	15.6±0.57^{cde}	6.0±0.00^{n}	13.3±0.57^{fgh}	7.0
L. monocytogenes EGD	14.3±0.57^{defg}	11.3±0.57^{ijk}	6.6±0.57^{mn}	11.0±0.00^{jkl}	10.
L. monocytogenes ATCC 7644	13.0±1.73^{fgh}	11.0±0.00^{jkl}	6.0±0.00^{n}	12.6±0.57^{ghi}	10.
Gram negatives					
E. coli ATCC 1858	7.6±2.88^{kl}	9.3±0.57^{op}	6.6±0.57^{mn}	6.0±0.00^{n}	6.
S. Typhimrium ATCC 14028	7.6±2.88^{kl}	6.6±0.00^{mn}	6.00±0.00^{n}	6.0±0.00^{n}	6.
Streptomycin (100mg/ml)	17.0	18.0	18.0	5.5	
DMSO	6.0	6.0	6.0	6.0	

*Means inhibition (mm) ± S.D of three replicates with different lowercase letters is significantly ($P<0.0$

4.2 Broth dilution assay

The MIC and MBC values obtained at 24 and 48 h using broth dilution assay for the two plant ethanol extract of *T. catappa* and *A. purpurata* are presented in Table 2. The MIC value of *T.catappa* extract against *S. aureus* 113, S.aureus 29213 strains tested showed 10 mg/ml at 24 h whereas MBC found to be 80 mg/ml irresspective of the strain and the value did not change at 48 h.Further MIC found for*L. monocytogenes V7* was 5 mg/ml. The MIC value of *A. purpurata* hexane extract showed 5 mg/ml and 10 mg/ml against *L. monocytogenes V7*and *E. coli* respectively.

According to (Shanhina, Samia, Sheikh, Rahmanullah, Syed (2007)*T .catappa* fruit methanol-choroform crude extract showed MIC 31.25µg/ml against *S. aureus*, *E. coli* and *S.* Typhimureum. These

MB

2

8

differences may be due to the differences of concentration of the crude extract, and solvent type used for the extraction.

The MBC values for all tested strains of *S. aureus* were found to be 80 mg/ml for *T. catappa* whereas *L. monocytogenes* it was 20 mg/ml for *A. purpurata* ethanol extract.

Table 2 The MIC and MBC values of plant extracts against food-borne pathogens

Plant	Name of the organism	Concentration (mg/ml) of MIC	
		24 h	48 h
T.catappa (ethanol)	S. aureus113	10	10
	S. aureus ATCC29213	10	10
	L. monocytogenes V7 (1/2a)	5	5
A.purpurata (ethanol)	L. monocytogenes V7 (1/2a)	5	5
	E. coli	10	10

MIC of *T.catappa* indicates activity within the range of concentrations tested (0.625 - 80 mg/ml), *A. purpurata* within the range of concentration tested (1.25 – 20 mg/ml).

4.3 Total phenol content

The TPC of ethanol extract of *T. catappa* was 81.54 ±1.28 mg/GAE/ g. However, (Abdulkadir, 2015) showed that TPC of the *T. catappa* fruit extract contains 117.10 (mg GAE/ g).

4.4 GCMS analysis

The GCMS chromatograph is illustrated in the Figure 1 and compounds eluted in the spectrum are presented in Table 3. GC-MS analysis of the study demonstrated that the major chemical compound of *T. catappa* ethanol extract yielded 31.86 %. The major compound identified was 2, 5-Furandione, 3 methyl, having molecular formul ae $C_5H_4O_3$ and the molecular weight 112. However none of the researches not yet identified the chemical composition of fruit of *T. catappa*. The antibacterial activity of the plant extracts might be attributed to the presence of bioactive plant compounds.

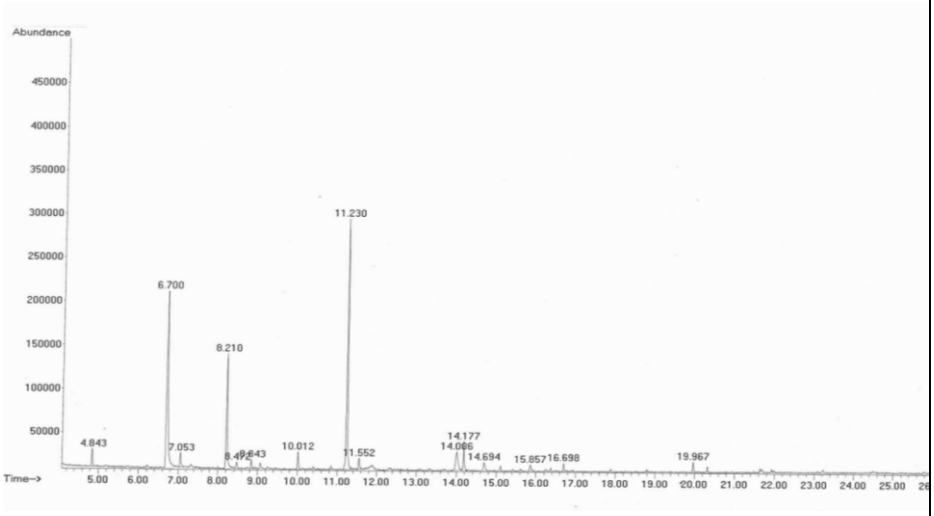

Figure 1 GCMS chromatogram of *T. catappa* (ethanol) crude extract

Table 3. GCMS analysis data of *T. catappa* (ethanol) crude extract

Peak*	Chemical Compound	% Percentage

4.84	2-Furancarboxaldehyde	2.18
6.70	2,5-Furandione, 3-methyl	31.86
7.05	2-Furalcarboxyadehyde	1.96
8.21	Furan	15.60
8.84	4,5-Diamino-2-hydroxypyrimidine	1.23
10.01	4H-pyran-4-one	2.24
11.23	2-Furalcarboxyadlehyde	29.33
11.55	3-hydroxythiophenol 4-mercaptophenol	1.46
14.00	Cyclophentanol	4.51
14.17	L-Glutamic acid 5-ethyle ester	3.49
14.69	D-allose	1.79
15.85	Methyl.beta-d-ribofuranoside	1.02
19.96	Hexadeconoic acid	1.27
	Total	97.90

*peak time in minutes

5. Conclusions, implications and significance

The present study was aimed to identify the antibacterial activity of the selected underutilized plants against food born bacteria. The MIC values for ethanol extracts of *T. catappa* and *A.purpurata were* ≤ 10 mg/ml for the tested bacteria. MBC of *T. catappa* was 80 mg/ml for *S. aureus 113* while 20 mg/ml for *L. monocytogenes* V7. The major chemical compound of the ethanol extract of *T. catappa* was 2, 5Furandione, 3 methyl (31.86 %). In conclusion, *T. catappa peri*-carp is a potential source of bio preservative and additional research and clinical trials are needed for the product development to strengthen the usage of *T. catappa*.

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Revised Abstract