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Identification of antimicrobial active components from dry Hainan *Morinda citrifolia* linn (Noni) fruit using GC-MS

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Abstract
Noni (*Morinda citrifolia* Linn.) fruit is a nutritional food with more potential health benefits for many years. In the present study, methanol solvent extract of dry noni fruit was obtained and its active components was investigated. Firstly, the extract showed antimicrobial activity against Escherichia coli, Streptococcus thermophilus, Staphylococcus aureus, Saccharomyces cerevisiae, and Candida albicans in disk diffusion assay with inhibition zone range (12.25, 12.31, 10.46, 10.77, 12.08 mm) at concentration of 860 mg/mL respectively. Then, twenty-one components were separated and identified in the extract by GC-MS. Among them, six were never reported in Noni plant. Finally, three novel components, 2-furan methanol, 2-furancarboxaldehyde, 5-methyl, and dihydro-3-(2H)-thiophen-one were obtained commercially. The dilution turbidity assay showed that they all exhibit antimicrobial activities against the above strains. Our data provides sufficient evidence of novel for its application in the food industry case of bio-preservatives and dietary sources for antimicrobial activity from the natural source.

Keywords: noni, antimicrobial, active components, bio-preservatives, fruit extract, gas chromatography

Introduction

Morinda citrifolia Linn, commercially known as 'Noni', is found grown in both, tropical and subtropical countries in the South Pacific islands, Asia i.e. Hainan, India, and South America i.e. Brazil. The Noni plant is an important traditional Polynesian medicinal plant and nutritional food for almost over 2000 years with more potential health benefits (Almeida, de Oliveira *et al*., 2019; West *et al*., 2018) [2-21] , Noni fruit itself is commonly consumed as a juice beverage, although leaves, flowers, bark, and root were also used in traditional medicine preparation (Ahmad *et al*., 2016) [1]. The diverse number of properties of the plant were identified, including immunostimulatory, antitumor, antidiabetic, anti-obesity, antibacterial, antiseptic, antifungal activities, and so on (Lin *et al*., 2017) [15]. Both *in viv*and *in vitro* studies indicate that the plant exhibits great use in alternative medicine for various illnesses (West *et al*., 2018) [21]. Counts of 160 phytochemicals compounds in Noni were identified and with major micronutrients all of which are phenol compounds, organic acids, and alkaloids (Lin *et al*., 2017 [15]. Solvent extracts of hexanoic, chloroform, ethyl acetate, alcoholic, and water obtained from Noni leaves, roots, fruits (juice), and seeds have shown the anti-bacterial or anti-septic activity and antifungal activity against many antibiotic susceptibility bacteria in West *et al*. (2018) [21]. Despite much research in past decades, there is still not much knowledge of the potent bioactive compounds extracted from the Noni plant fruit (Jainkittivong *et al*., 2009) [9]. This study show the antimicrobial effect of the dry Noni methanol sol vent extract and *in vitro* study to novel active components present in the crude extract identified through GC-MS analysis.

Materials and Methods

Plant Materials Procurement

Dried Noni (*Morinda citrifolia* Linn) fruit slices were purchased from five finger mountain Li Miao Chinese medicine health and wellness research institute, Wukshan city; Hainan, China in May 2019. Plants were grown under tropical conditions (mean annual temperature: 20-35°C, minimum temperature: 12°C). The ripe fruits (light dull, whitish, soft, and fetid pulps; 5-10 cm long and 3-4 cm approximately) were harvested and washed clean. Pulps were sliced and dried in sterilized heat pump dryer chambers at a temperature of 50 to 60°C for a day. Net weight 161 grams of dry fruit slices were stored in a sealed desiccator.

Test strains and Chemical reagents

Bacteria strains *S. thermophilus*, *S. aureus*, and *E. coli* and fungal strains *C. albicans* and *S. cerevisiae* supplied from State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology (Table 1). Yeast extract, tryptone was purchased from (Oxoid Ltd, Shanghai) and agar powder (Sangon Biotech Co., Ltd, Shanghai). D-glucose, sodium chloride (>99.5%), and methanol (99.5%) (Shanghai Titan Co., Ltd, Shanghai). Pure Components of 2-furan methanol (99%), furfuryl alcohol (99%) (Adamas Reagent Co., Ltd, Shanghai), and dihydro-3-(2H)-thiophen-one (99%) (Budweiser Technology Co., Ltd, Shanghai). Ampicillin Na and kanamycin sulphate (Sigma Aldrich Trading Co., Ltd, Shanghai). Reagents used in the experiment were of either analytical grade or chromatographic grade.

Table 1: Microbial test strains incubation medium and optimum condition. Microbial test strains medium.

Strain	Strain name	Culture	Origin
Bacterium	*Escherichia coli*	LB, 37°C	China industrial microbial species preservation and Management Centre
	Streptococcus thermophilus	LB, 37°C	China industrial microbial species preservation and Management Centre
	Staphylococcus aureus (ATCC2600)	LB, 37°C	China industrial microbial species preservation and Management Centre
Fungal	*Candida albicans* (CMC96001)	YPD, 37°C	China industrial microbial species preservation and Management Centre
	Saccharomyces cerevisiae (BY4741)	YPD, 30°C	Invitrogen

(YPD) Yeast extract peptone dextrose medium, (LB) Luria-Bertani culture base/ lysogeny medium.

Preparation of Noni fruit Methanol (NFM) extract

Blend Noni dry fruit grounds, weight 40 g was reconstituted in 400 mL of methanol solvent and preserved in a cooler 4°C for 120 hrs. The sample was centrifuged, obstructing precipitants then supernatant was filtered through organic filters and hydrolyzed with freeze Vacuum-Pressure Centrifuge at 55-60°C. Powder extract was then stored in tight dark sealed desiccator for future use.

NFM extract for antimicrobial inhibition zone in disk diffusion assay

Strains *E.coli, S. thermophilus*, and *S. aureus* were inoculated in LB aqueous liquid medium (1% tryptone, 1% sodium chloride, and 1.5% yeast extract) (w/v) ; at 37°C in 24 hrs with agitation. *S. cerevisiae* and *C. albicans* was also incubated in aqueous medium (1% yeast extract, 2% tryptone, and 2% glucose) (w/v) at 30°C and before transferred to solid medium of 1.5% agar solid petri dish culture. NFM extract attain concentrations of 860 mg/mL and 430 mg/mL, 30 µL volume sample was condensed onto 6 mm sterilized filter paper discs before placement onto the microbial surface agar assay. Administered positive controls (PC) of kanamycin sulphate (4.2 mM), ampicillin Na (100 mM), and blank control (BC) of 10% (v/v) methanol were sampled and incubated for 24hrs. The inhibition zone was measured using a 3202 Dial Caliper 0.8-inch instrument (No.1202) Starrett, the USA around the disk (diameter in mm). All test samples of the test were conducted in triplicates.

Analysis of NFM extract compounds by GC-MS

NFM extract was analyzed by Agilent system 6890 gas chromatograph coupled to an Agilent 5975 quadrupole mass selective detector (EI) (Agilent Technologies, Santa Clara, CA). GC-MS was equipped with an HP-5 column (30 m long × 0.25 mm ,i.d., with 0.25 µm film thickness). Injector temperature at 250°C with an injection volume of 1 µL. The mass spectrometer was operated in scan mode (starting after 5 min, mass range 50–550 AMU). Column flow was held constant at 1 mL He/min, with the inlet temperature of 280°C. Interface temperature was at 250°C and quadrupole at 200°C. Tenfold dilution of NFM extract injection volume of 1 µL, was injected in split-less mode, oven temperatures programmed at 60°C for 2 min, gradually raised with a gradient of 5°C/min until 280°C for 20 mins, then gradient was set to initial, 20 °C /min for 3 mins. Mass spectral data were analyzed with commercial NIST 05 Mass Spectral Library or custom internal database generated from authentic compounds or internet shared MCF derivatized metabolites in-house MS library. Data files were generated in AIA format using MSD Chem Station (Agilent Technologies, Santa Clara, CA) and aligned with quantitative and qualitative data using MET-IDEA software (The Samuel Roberts Noble Foundation, Ardmore, OK). The extracted data were normalized to the internal standard and dry weight of biomass. The clustered Heat map was generated with Gene cluster 3.0 and visualized by Tree View 1.1.6r4 (Carl Icahn Laboratory, Princeton, NJ).

Minimum inhibitory concentration (MIC) broth dilution assay of active components against strains

Strains were inoculated into 5 mL of complete medium in a rotary shaker for 24 hr. Growth was assessed by performing cell counts with an ultraviolet spectrophotometer at OD_{600}. All strains were diluted to OD_{600} = 0.1(Dynamica DNA master spectrophotometer, UK), then sampled into a culture tube at 200µL in triplicates. Serial dilution concentrations of all three active components of 2-Furanmethanol; 2-Furan-carboxaldehyde, 5-methyl and Dihydro-3-(2H)-thiophen-one with respective concentrations of 2-30 mg/mL treatment were sampled into each strain broth (OD_{600} = 0.1) and a control, in triplicates. The samples were then incubated for 24hrs with rotation. Results of turbidity visual in broth dilution method implemented by the CLSI (Clinical Laboratory Standards Institute) were conducted. Records of active component corresponding inhibitory concentration obtained with equation (1) of all test samples with turbidity visual test and also OD_{600} values. A half microbial inhibitory concentration (MIC_{50}) of each active components was analyzed by strain broth OD_{600} values dose-response by GraphPad prism.

Statistical Analysis

All the experiments were performed in triplicates and results were expressed with mean values ± standard deviation. One-ANOVA with and Tukey's test was performed to test the differences between test and control groups ($P<0.05$) in all test results. Statistical analyses were performed using GraphPad Prism Version 8.0.2.

Results and discussions

Yield of dry NFM extract

A dry weight of 10.3 g per 40 g powder extrapolated a 25.8% recovery rate. A NFM extract stock was stored in sealed desiccator under dry condition for future use. Samples were preserved in the freezer 4°C after use each antimicrobial test preparation.

The inhibition zone analysis of NFM extract on strains

NFM extract inhibition zone on the agar assay according to different concentrations administered with the positive control (PC) and blank control (BC) shown in (Figure 1). Inhibition zone ranged as follows: *S. thermophilus* (12.31 ± 0.40) mm > *E. coli* (12.25 ± 0.42) mm > *S. cerevisiae* (12.08 ± 0.61) mm > *C. albicans* (10.77 ± 0.87) mm and *S. aureus* (10.46 ± 0.84) mm in diameter at concentration 860 mg/mL , At half concentration of 430 mg/mL administered; strain inhibition zone range from *S. cerevisiae* (9.91 ± 0.73) mm > *C. albicans* (9.32 ± 0.69) mm > *T. streptococcus* (8.65 ± 0.45) mm > *E. coli* (8.64 ± 0.79) mm and *S. aureus* (8.08 ±

0.78) mm. Speculation was the NFM extract exhibited effective antimicrobial activity to strains due to potential active components present in the NFM extract.

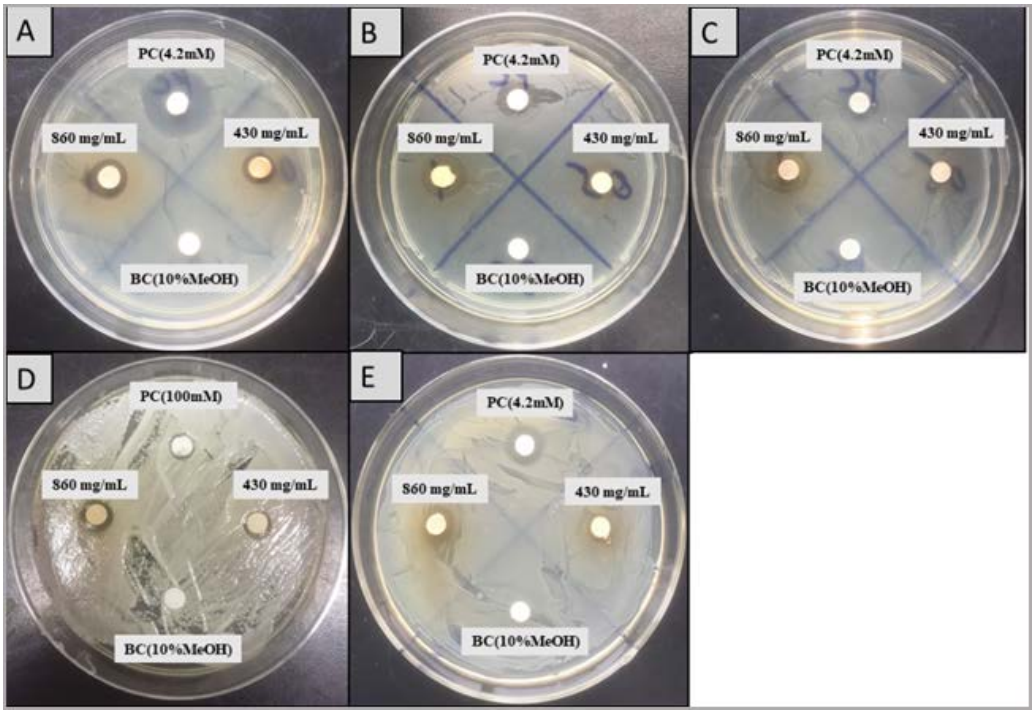

Fig 1: Noni fruit methanol (NFM) extract concentration with controls inhibitory zone on agar assay test against strains; A) *E. coli*, B) *S. thermophilus*, C) *S. aureus*, D) *C. albicans, and* E) *S. cerevisiae*. Disk diffusion assay of noni fruit methanol (NFM) extract concentrations: 860mg/mL and 430mg/mL inhibitory zone on agar assay test against strains. Administered positive controls (PC) of kanamycin sulfate (ɤ = 4.2 mM) and ampicillin Na (§=100 mM). Blank control (BC) : 10% (v/v) methanol. Inhibition zone of NFM extract above 50% diameter of (PC) is considered effective. Statistical analysis was performed one-way ANOVA and Tukey's test was performed to test the differences between test and control groups in multiple comparison

Separations and identification of active components of NFM extract

Total of twenty-one components were separated by gas chromatograph and eleven were identified from the NFM extract by mass spectrometer (Figure 2). Graph show peak number (P#) ranked of active components separated by gas chromatography. P#1: 2-furan carboxaldehyde,5-(hydro-methyl); P#2: furfural; P#3: 4H-pyran-1-one,2,3-dihydro-3,5-dihyroxy-6-methyl; P#4: N-hydroxymethyl acetamide; P#5: octanoic acid; P#6: furyl-hydroxymethyl ketone; P#7: 2-furanmethanol; P#8: pentanoic acid,3-methyl; P#9: 2-furancarboxaldehyde,5-methyl; P#10: 2,4-dihydroxy-2,5-dimehtyl-3(2H)-furan-3-one and P#11: dihydro-3-(2H)-thiophen-one. Peak analysis of retention time and abundance degree (m/z) of composition detail seen in (Table 2).

Comprehensive research review eliminates five used active components above and was confirmed to have been reported for being extracted from other plant parts including Noni plant for studies. The active component of peaks; P#1: 2-furan carboxaldehyde, 5-(hydro methyl) was reported from the ethanol extract of *Diospyros celebica* bark (Rose wood) with antimicrobial activity (Mohy El. Din *et al*., 2018) [17] with P#8: pentanoic Acid. Ge *et al*., (2018) [6] investigated similar component in methanol extract of bayberry, rich in rare bio medicinal activities in Chinese traditional medicine which also containing P#3: 4H-pyran-1-one,2,3-dihydro-3,5-dihyroxy-6-methyl, hexanoic acid , and 9-octadecenmoic acid (E). Kahn *et al*. (2018) [13] used Homalium Zeylanicum bark and leaf extract and reported anti-oxidant principle responsible for hematopoietic activities for liver protection which contain P#2: furfural, hexanoic Acid, and 9-octadecenmoic acid (E). Both components give subcritical hydrothermal treatment on palm oil and in Securidaca inapperdiculata Harsk fatty oil for medicinal potentials, suppressing pathological functions of fibroblast (Jiang H *et al*., 2018; Jiang K *et al*., 2018) [10, 11]. Component P#5: octanoic acid and P#8: pentanoic acid, 3-methyl in Mathan Tarilan (*mathan tarilan, panchai ennai*) were used to treat diabetic foot ulcer (Lee *et al*., 2019) [14]. Garba *et al*. (2018), Ghate *et al*., (2016), and Efavi *et al*., (2018) [5, 7, 4] reported P#5: octanoic acid and hexanoic acid components for anti-cancer, anti-inflammatory properties in sundew plant and such agents showing an ethnopharmacological approach for new drug discovery. Jiang H *et al*. (2018) [10] exploited rosewood (*Dalbergia stereson*) with the same components for potential prospects in fields of biomedicine, cosmetic, and skincare products. Mohy El. Din SM & Mohyeldin MM (2018) [17] reported antifungal with extract of four brown seaweed solvent extract with anti-inflammatory activities with component P#5: octanoic acid appearing prominently. In both Tao *et al*. (2018) [19] and Tang *et al*. (2018) [18] reports, there were anti-inflammatory, antitumor, antimicrobial, antioxidant, antiallergy, immunomodulating, and antibacterial activity in Traditional Chinese Medicine (TCM) with P#5: octanoic acid presence amounts other components for studies in bamboo leaf. Anticancer with components from Rhizome of Tectaria cicutari extract in traditional medicine for various disorders which lead to investigation on the mechanism for cancer study reported similar components above to be involved (Karade *et al*., 2018) [12]. Mawa *et al*. (2019) [16]

reported the same components present in *Leca macrophylla* (Roxb) *ex Hornem* root, with functional effect on pancreatic necrosis and Zhao *et al*. (2015) [23] reported antibacterial activities, mechanisms of sugar fatty acid esters against bacteria. Remaining six components; P#4: N-hydroxy-methyl-aceramide, P#6: furyl-hydroxy-methyl-ketone, P#7: 2-furanmethanol, P#9: 2-furan-carboxaldehyde,5-methyl, P#10: 2,4-dihydroxy-2,5-dimehtyl-3(2H)-furan-3-one and P#11: dihydro-3-(2H)-thiophen one were distinguished as novel potential unused active components.

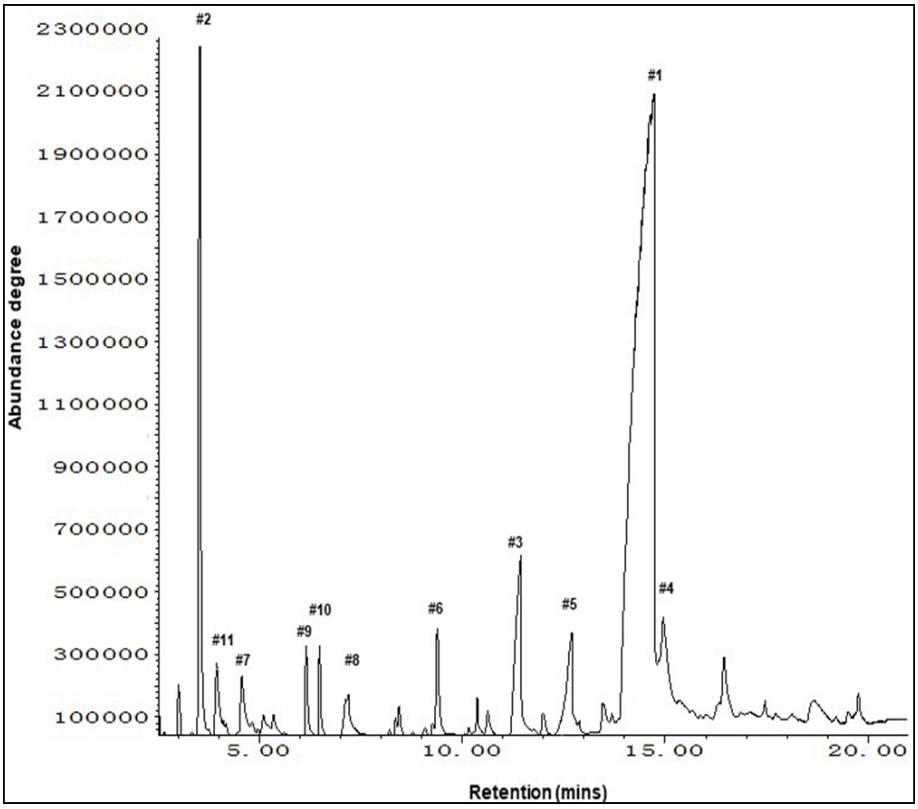

Fig 2: GC-MS tree graph analysis of NFM extract. Peaks (#) marked active components, peak #1 to peak #11 ranked according to active component composition properties in the NFM extract. Vectors of retention time (mins) on abundance degree.

Table 2: Active components identified in NFM extract from mass spectrometer.

Peak #	R.T (mins)	C.A (%)	T.R (%)	Active component name
#1	14.718	100	53.23	2-Furancarboxaldehyde, 5-(hydro-methyl)
#2	3.540	14.77	7.86	Furfural
#3	11.434	8.35	4.44	4H-Pyran-1-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl
#4	14.954	5.83	3.10	N-Hydroxy-methyl-acetamide
#5	12.703	4.90	2.60	Octanoic Acid
#6	9.393	3.07	1.63	Furyl-hydroxymethyl Ketone
#7	3.957	2.85	1.51	2-Furanmethanol
#8	7.193	2.12	1.12	Pentanoic Acid, 3-methyl
#9	6.163	2.08	1.10	2-Furancarboxaldehyde, 5-methyl
#10	6.492	1.81	0.96	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
#11	4.578	1.78	0.94	Dihydro-3-(2H)-thiophen-one

GC-MS analytical identification of active components in NFM extract. R.T - Retention time; C.A (%) - Corrected area share (%); T.R (%) - Total ratio (%).

MIC Study of 2-Furanmethanol, 2-Furan-carboxaldehyde, 5-methyland Dihydro-3-(2H)-thiophen-one on tested strains

Six novel components in the study has reported with usual characteristics seen in (Table 3). Three of high pure grade components, P#7: 2-furanmethanol, P#9: 2-furan-carboxaldehyde, 5-methyl, and P#11: dihydro-3-(2H)-thiophen-one; were commercially available and have been already used in the food industry as flavorings.

MIC of active components concentration administered seen via turbidity observation seen in (Table 4) and with the formulation of equation (1) for each strain microbial inhibitory in percentage.

$$\text{Strain Inhibition } (\%) = \left(\frac{OD_{600}(Treated) - OD_{600}(Untreated)}{OD_{600}(Untreated)}\right) \times 100 \quad (1)$$

MIC corresponding concentrations of Component P#7: 2-furanmethanol with negative turbidity on *S. aureus*, *S. cerevisiae* were at 20 mg/mL and *E.coli* , *S. thermophilus* and *C. albicans* at 25 mg/mL for the MIC.

Component P#9: 2-furan-carboxaldehyde, 5-methyl against *C. albicans* strain negative turbidity at 15 mg/mL; *E.coli* and *S. thermophilus* at 10 mg/mL and 5 mg/mL for *S. aureus* and *S. cerevisiae*. Component P#11: dihydro-3-(2H)-thiophen-one with on *E.coli* and *S. thermophilus* at 20 mg/mL and *S. aureus, C. albicans,* and *S. cerevisiae* with 25 mg/mL for the component broth visual analysis. Optical density (OD_{600}) readings was acquired of each samples from the broth dilution test. MIC_{50} of each active components was further generated through GraphPad prism analysis, extrapolating concentrations of all three active components against test strains. Component P#7: 2-furanmethanol MIC_{50} values as listed *S. cerevisiae* = 16 mg/mL> *C. albicans* = 12 mg/mL > *S. aureus* = 11 mg/mL > *S. thermophilus* = 9.3 mg/mL > *E.coli* = 4.4 mg/mL , Component P#9: 2-furanmethanol with *E. coli* = 4.2 mg/mL > *S. thermophilus* = 3.9 mg/mL > *S. cerevisiae* = 3.1 mg/mL > *C. albicans* = 2.3 mg/mL > *S. aureus* = 1.7 mg/mL , P#11: dihydro-3-(2H)-thiophen- one with *S. aureus* = 13 mg/mL > *S. cerevisiae* = 9.2 mg/mL > *E. coli* = 9.1 mg/mL> *C. albicans* = 8.4 mg/mL and *S. thermophilus* = 7.8 mg/mL , Ordinary One-way ANOVA and Tukey's -test values and significantly different among means of ($P<0.05$).

Result show the NFM extract with antimicrobial effect also contains three new active components 2-furanmethanol, 2-furan-carboxaldehyde,5-methyl, and dihydro-3-(2H)-thiophen-one contributing to the inhibition of microbials. Studies show all were used in food preservatives and flavorings and present in natural food products such as in dry noni fruit. Speculation of antimicrobial of NFM may contribute a lot in replacing the use of synthetic preservatives which are harmful to humans to differing degrees. Causes of poisoning, allergic reactions, teratogenicity, and even cancer with uprising concerns to new synthetic preservatives used in food (Zhang *et al*., 2016) [22], yet may cause fewer side effects when using natural herbs like Noni. Studies show three active components occur naturally in dry noni fruit and lab producing these compounds can be explored and developed as starter cultures for fermented food and may also be used to prevent microbial contamination of food. The plant may serve its purpose in antimicrobial, inhibiting both fungal and bacterium strains to improve food quality and prolong shelf-life with considerable potential for the utilization of natural antimicrobial from plants in foods. The GC-MS analysis and partitioning process of the extract is limited for this study yet further test and analysis could be with other technology to further verify the presence of antimicrobial organic volatile active components present in the noni dry fruit plant shortly and also be used in other studies.

In this study, NFM extract possesses both antibacterial activities against *E.coli, S. thermophilus, S. aureus* and antifungal activity against *C. albicans* and *S. cerevisiae*. Twenty-one components were separated via gas chromatography and eleven were identified by mass spectrometer analysis. Among these components, five were already used in past studies, leaving six novels and unexploited. Broth dilution shows that all three commercially available components including 2-furan methanol, 2-furan-carboxaldehyde,5-methyl, and dihydro-3-(2H)-thiophen-one exhibit antimicrobial activity against the tested strains. Further studies *in viv*and clinical research are needed to explore their antibacterial and antifungal effect in the future.

Table 3: Preview on six novel active components in NFM extract.

Peak # - Ret. Time (mins) and active component name	Chemical characteristics and product category use
#4-14.95 mins: N-Hydroxymethyl acetamide. CAS: 625-51-4. Molecular Weight: 89.09 g/mol. Molecular Formula: $C_3H_7NO_2$, Appearance: white to light yellow crystalline powder, Melting Point: 81 – 83 °C Storage: 20ºC Room temperature, sealed, dry.	Air & Water Reactions: Soluble. Sensitive: Hygroscopic. Uses: Antiseptic, disinfectant for surgical instruments. Hazard Identification: Irritant.
#6-9.39 mins: Furyl-hydroxymethyl ketone. CAS: 17678-19-2.Molecular Weight:126.11g/mol. Molecular Formula:$C_6H_6O_3$. Appearance: Off-White to Light Beige Solid. Melting Point: 81 – 83 °C. Storage: Hygroscopic, -20 °C Freezer. Under inert atmosphere.	Air & Water Reactions: Chloroform (Slightly), DMSO (Slightly).Soluble in water and Alcohol. Uses: Reagent in preparation of 3-iodocoumarins and quinoxalines used in dyes and pharmaceuticals. Hazard Identification: Toxic.
#7-3.95 mins: 2-Furanmethanol. CAS: 98-00-0. Molecular Weight: 98.1g/mol. Molecular Formula: $C_5H_6O_2$, Appearance: Pale Yellow to Orange Oil/ Clear Colourless. Melting Point: 170 °C(lit.). Storage: Refrigerator/ Room temperature.	Product Categories: Air & Water Reactions: Chloroform (Sparingly), Methanol (Slightly), Slightly soluble in water. Uses Furans; alcohol Flavor; fine chemical; fine chemicals; industrial-grade; additives; pharmaceutical raw material. Hazard Identification: Toxic, Irritant. Description: Solvent and manufacturing of wetting agents, resins. The basic raw materials of its manufacturing are waste vegetable materials; rice hulls, sugar cane bagasse, oat hulls, or corncobs. Found in coffee, tea, wheat bread, crispbread, soybean, cocoa, rice, potato chips, and other sources. It is flavoring.
#9-6.163 mins: 2- Furancarboxaldehyde,5-methyl. CAS: 620-02-0. Molecular Weight: 110.11g/mol. Molecular Formula: $C_6H_6O_2$, Appearance: Yellow to Orange Oil. Melting Point: 187-189 °C(lit.). Storage: Hygroscopic, Refrigerator, under inert atmosphere 2~8 °C.	Product Categories: Building Blocks; Miscellaneous. Air & Water. Reactions: Chloroform, Methanol (Slightly). Uses: use daily flavor, food flavor, cosmetic. Hazard Identification: Toxic, Irritant. Description: Common chemical reagents used chemical reactions and organic synthesis. Polycyclic pyridazine-based derivatives are synthesized with this compound to form antimicrobial agents and are also used in the preparation of anti-inflammatory diaryl-pentanoid analogs.
#10-6.492 mins: 2,4- Dihydroxy-2,5-dimehtyl-3(2H)-furan-3-one. CAS: 10230-62-3. Molecular Weight: 144.12 g/mol. Molecular Formula:$C_6H_8O_4$, Appearance: N/A. Melting Point: 281.2±40.0 °C(Predicted).	Product Categories: Heterocyclic Organic Compound. Description: A flavor compound that occurs in fruits such as pineapple, strawberry and mango.

#11-4.578 mis: Dihydro-3-(2H)-thiophenone. CAS: 1003-04-9. Molecular Weight: 102.15g/mol. Molecular Formula: C_4H_6OS. Appearance: N/A. Melting Point: 175.2±23.0 °C. Storage: 2-8 °C.	Product Categories: Building Blocks; Miscellaneous. Air & Water Reactions: Air Sensitive. Uses: Used for blending soft drinks, beverage, meat products, confectionery, and milk product flavors. Hazard Identification: Causes skin irritation with serious eye irritation. Description: Heterocyclic nonaromatic ketone with garlic meaty, green vegetable, buttery odor. Present in cooked beef, coffee, roast filbert, and roasted peanut. Used as a food flavoring agent. A valuable organic substance key that opens the synthetic access to numerous derivatives of thiophene is a useful research chemical with an endogenous metabolite.

Descriptive property of novel active components in NFM extract with chemical and physical properties.

Table 4: Broth dilution MIC study of active components identified in NFM extract.

Strain	UT	T		T		T	
	Turbity	P#-7	Turbity	P#-9	Turbity	P#-11	Turbity
E.coli	(+)	25	(-)	10	(-)	20	(-)
S. thermophilus	(+)	25	(-)	10	(-)	20	(-)
S. aureus	(+)	20	(-)	5	(-)	25	(-)
C. albicans	(+)	25	(-)	15	(-)	25	(-)
S. cerevisiae	(+)	20	(-)	5	(-)	25	(-)

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Declarations

Conflict of Interest
The authors declare no conflict of interests regarding the publication of this paper.

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