# ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF *Cassia fistula* SEED AGAINST SPOILAGE BACTERIA OF SOYMILK

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***Abstract***
*Plants derived bioactive compounds can serve as an alternative to synthetic antibiotics in effective control of bacterial infections. This study was carried out to investigate the antimicrobial activity of ethanol extract of Cassis fistula seed against spoilage bacteria of soymilk and its application in shelf life extension. The spoilage bacteria were isolated using culture dependent method and identified by determining their gram reaction and biochemical characteristics. The agar well diffusion method was employed to determine the antimicrobial activity of the extract. Minimum inhibitory concentration (MIC) and the minimum bacterialcidal concentration (MBC) of the extract were assessed using the tube dilution method. Sensory evaluation of supplemented extracts and shelf life study under refrigeration and room temperatures were investigated using standard procedures. The phytochemicals analysis of extract showed the presence of alkaloids, flavonoids tannins, saponins and cardiac glycosides. The results obtained revealed that microorganisms such as Serratia marcescens, Proteus vulgaris, Pseudomonas aeruginosa, Proteus mirabilis and Klebsiella pneumonia were associated with the spoilt soymilk. The extract significantly ($P \leq 0.05$) inhibited Serratia marcescens, Proteus vulgaris, Pseudomonas aeruginosa, Proteus mirabilis and Klebsiella pneumonia. However, the MBC values for Serratia marcescens, Proteus vulgaris, Pseudomonas aeruginosa, Proteus mirabilis and Klebsiella pneumonia were 50.0±0.2, 25.0±0.2, 12.5±0.1,6.25±0.2, 6.25±0.2 mg/ml respectively. This study revealed that the extract possesses antibacterial activity against spoilage bacterial of soy milk and can be used to extend its shelf life.*

**Keywords:** Ethanol extract, *Cassia fistula* seed, antimicrobial, agar well diffusion, sensory evaluation, shelf life extension

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## 1. INTRODUCTION

With recent global documented reports on the incidences of resistance shown to antibiotics by bacteria, coupled with the currently reported occurrences of multi antibiotic resistant bacteria encountered in the treatment of microbial infections, there is urgent need to search for natural alternative remedy which is very effective, less expensive, non toxic with relative scanty side effects. Herbs, spices and their antimicrobial constituents are generally regarded as save (GRAS) (Gupta, 2010). Previous documented research had confirmed the ability of solvent extracts from morphological organs of plant to exhibit anti-bacterial activity against gram positive and gram negative bacteria , mould and yeast (Issabeagloo *et al*, 2012; Ismail *et al*, 2012). Luximon *et al*. (2002) and Gupta (2010) reported that the anti-inflammatory and antimicrobial potentials of plant extracts are due to the possession of important secondary metabolites and phytochemical compounds which are responsible for structural diversity and biological functions (Pandey *et. al.*, 2011). Such compounds include polyphenols which can inhibit extracellular enzymes or the multi-drug resistant pump of certain bacteria, exhibits antibacterial adhesion activity which is a crucial factor in determining adherence to mucosa surface. (Puuponea-Pimia *et al.*, 2004; Prashanth *et al.*, 2006; Subramanion *et al.*, 2011; Savoia, 2012) In addition, previous studies had confirmed that the efficiency of antibacterial activity of plant extract is partly dependent on the extracting solvents (Cowan, 1999; Gupta, 2010; Chitra *et al.*, 2012). According to Gupta *et al*. (2008) the presence of essential oil in medicinal plants equally confers inhibitory activity which can be exploited in the food industries as botanical

preservation. Apart from this, plant extracts can be applied in the food industries as flavor and aroma enhancers which are derived from the phytochemical compounds present in them such as alkaloids, flavonoids, tannins saponins and cardiac glycosides (Melvin *et al.,* 2002). Natural additives in most cases are believed to be healthier, confer added value (bioactivity, nutraceutical) and with array of other functions in the food, (Rasooli, 2007; Tiwari *et al.*, 2009; Brewer, 2011; Pillai and Ramaswamy, 2012).

*Cassia fistula Linn*. (Cassia) belongs to the family Caesalpiniaceae and it is commonly known as "Indian Laburnum" (Bhalerao and Kelkar, 2012). *C. fistula* plant parts are known to be important source of secondary metabolites, such as phenolic compounds with potent antibacterial, antifungal, anti-inflammatory and antioxidant properties (Gupta, 2010). Ethanol extract of this plant has been reported to be non toxic and hence can be utilized for pharmaceutical formulations (NISCIR, 2007).

Soymilk is a water extract of soybeans and consists of high content of water soluble protein, Carbohydrate and oil. It is a cheap source of plant protein and widely consumed by low income people in Nigeria because it is not expensive and easily produced with low level technology by almost every house hold in Nigeria with no knowledge of aseptic rules resulting in contamination by food borne microorganism. It is a good medium for growth of microorganisms which makes it easily susceptible to microbial spoilage coupled with short shelf life. Protection of food from microbial or chemical deterioration has traditionally been an important concern in the food industry. Chemically synthesized preservatives or artificial preservatives have been classically used to decrease both microbial spoilage and oxidative deterioration of food (Gould, 1995) and also prevent contamination of finished products. However, in recent years, consumers are demanding partial or complete substitution of chemically synthesized preservatives due to their possible adverse health effects and thus require an alternative with significant less risk. This fact has led to an increasing interest in developing more "natural" alternatives in order to enhance food safety and shelf-life (Roller, 1995).

This present work is intended to investigate the antimicrobial activity of ethanol extract from seed of *Cassia fistula* and its application in shelf life extension of soymilk.

## 2. MATERIALS AND METHODS

### 2.1 Sample collection

*Cassia fistula* fruit pods were collected from the Botany department of the Lagos state University (LASU) and from the Institute of Agricultural Research and Training, MOOR plantation Ibadan, Oyo state, Nigeria. The plant was authenticated as *Cassia fistula* by Professor E.A. Ayodele (Plant taxonomist) of the department of Botany University of Ibadan. The pods were cut opened and the seeds were washed, shade-dried for 2 weeks and were coarsely-milled with an electric blender and stored in a desiccator for further use.

### 2.2 Preparation of ethanol extract of *C. fistula* seed

The method described by Subramanion *et al*. (2011) was used for extraction of the seeds. One hundred and fifty grams of the dried seed powder was extracted by the addition of 80% (400 ml) ethanol with intermittent stirring for one week. It was filtered with muslin cloth and evaporated under reduced pressure using the rotary evaporator. The extract was further evaporated at 40°C in an electric oven to form paste and stored in stopper glass bottles at room temperature for further use.

### 2.3 Isolation and identification of soymilk spoilage bacteria

This was carried out according to the method described by Mbajiuka *et al.* (2014) Samples of soymilk were collected from vendors or hawkers at Agbowo street in Ibadan, Nigeria and were allowed to spoil by keeping it on the laboratory bench for 48h. The spoilt soymilk was serially diluted and 0.5ml of $10^{-4}$ dilution was plated differently on MacConkey agar, mannitol salt agar and Nutrient agar and

incubated for 24-48h at $37^0$C. The plates were examined for microbial growth and the pure cultures obtained were maintained in tryptone soy broth supplemented with glycerol in a refrigerator at $-80^0$C prior to use. Identifications of the isolates were carried out by considering their Gram reaction and biochemical characteristics and with reference to Bergey's manual of systematic bacteriology.

**2.4 Antimicrobial activity**

The antimicrobial activity of the plant extract was investigated using the agar well diffusion technique as described by Ahmad and Beg (2001).

***2.4.1 Preparation of inoculums***

The test bacteria were sub cultured at $37^0$C for 24h and the pure cultures obtained were washed in phosphate buffer (0.05M pH7.0) and transferred into normal saline. One hundred µl of the suspension containing$10^6$ CFU/ml of bacteria was used to seed Muller Hilton agar plates and wells were aseptically made on the agar plates using 5mm cork borer. Different concentrations of extract (One hundred microlitres (100 µl) were dispensed into the wells with the aid of a micro liter pipette and allowed to diffuse at room temperature for two hours. One hundred (100) µg/ml of Ciprofloxacin was dispensed at the respective well meant for control and the plates were incubated at 37°C for 24hr. The antimicrobial activity of the plant extract against the spoilage microorganisms was determined by taking measurements of diameter of zones of inhibition and expressed in millimeters.

**2.5 Qualitative and quantitative determination of phytochemicals:**

The screening for the phytochemical constituents present in the seed extract of *C. fistula* was carried out according to the procedures described by Ajaiyeoba *et al.* (2015) while the quantitative determination was carried out according to the procedures described by Marcano and Hasenawa (1991). The analyses were carried out at the Department of Pharmacognosy University of Ibadan, Nigeria.

***2.5.1 Determination of total flavonoids***

Half (0.5) ml 2% $AlCl_3$ methanol solution was added to 0.05 ml of the sample solution and allowed to stand for 1hour at room temperature. The absorbance was measured at 420 nm wavelength ($A_{420}$) with appearance of yellow colour indicating the presence of flavonoids. The flavonoids content was calculated as mg/g quercetin from a standard calibration curve.

***2.5.2 Tannins***

One gram of the sample was extracted with 25 ml of solvent mixture (Acetone: Glacial acetic acid). The solution was filtered and the absorbance measured at 500 nm. The concentration of tannin was read from a standard curve. A standard graph obtained with 10, 20, 30, 40, 50 mg/100g of tannic acid was constructed to read the concentration of tannin taking into consideration the dilution factor.

***2.5.3 Alkaloids***

One gram of the sample (W) was weighed into 20 ml 10% acetic acid in ethanol in a container. The solution was shaken, allowed to stand for 4hr and filtered. The filtrate was evaporated to about a quarter of its original volume and one drop of concentrated ammonia was added. The precipitate formed was filtered through a pre-weighed filter paper(W1) and dried in an oven at $60^0$C and weighed to a constant weight (W2) and the % alkaloids was calculated by the formula: %Alkaloids = (W2 – W1) × 100

***2.5.4 Saponins***

One gram of the sample was weighed into 5 ml 20% ethanol in a container and placed in a water bath at $55^0$C for 4hours.The filtered residue was washed with 20% ethanol twice and the extract was reduced to about 5 ml in the oven and 5 ml of petroleum ether was added. The pet ether layer was discarded and 3 ml of butanol was added. The mixture was washed with 5 ml 5% sodium chloride and the butanol layer poured into a pre-weighed Petri dish then placed in the oven to evaporate to dryness and weighed.

### *2.5.5 Cardiac glycosides*

One gram of the sample was extracted with 40ml of water and placed in the oven at $100^0$C for 15 minutes. One ml of the extract was added to2 ml glacial acetic acid containing one drop of $FeCl_3$ in1 ml of concentrated $H_2S0_4$. The absorbance of the resulting solution was measured at 410 nm.

### 2.6 Determination of MIC and MBC

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were estimated for each of the test bacteria in triplicates. Double fold dilution of the extract was carried out to obtain concentrations of 3.125 mg/ml, 6.25 mg/ml,12.5 mg/ml, and 25.0 mg/ml in different test tubes containing 1 ml of nutrient broth. One hundred µL of each of the test bacteria from a 24 hour-old culture (containing $10^6$ CFU/ml (0.5 McFarland's standard) was used to inoculate the tubes differently and incubated at 37ºC for 24h A tube containing nutrient broth only was differently seeded with each of the test bacteria and incubated at the same temperature and time, this served as the control. The tubes were examined for bacterial growth based on turbidity. The minimum inhibitory concentration is the lowest concentration that completely inhibits the bacterial growth. To determine the MBC, one ml of broth was collected from tubes, which did not show any growth and inoculated on sterile nutrient agar by streaking. After incubation the concentration at which there was no visible growth, was considered as the minimum bactericidal concentration (Doughari, 2006). MBC was defined as the lowest extract concentration at which 99.9% of the bacteria was killed. Each experiment was repeated twice.

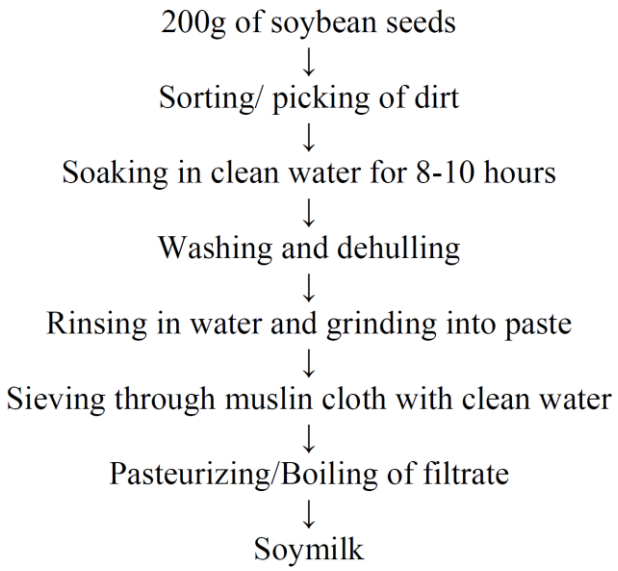


**Figure 1:** Flowchart for soymilk production (Lee *et al.*, 1990)

### 2.7 Treatment of soy milk

Two hundred ml of the prepared soy milk was filtered and dispensed separately into six 500ml Erlenmeyer flasks labeled $A_1$, $A_2$, $A_3$, $A_4$, $A_5$ and $A_6$ respectively. 1% ethanol extract of *Cassia fistula* seed was added to flask $A_1$, while 10, 20, 30, 40% were added to flasks $A_2$, $A_3$, $A_4$, $A_5$ respectively. Sample $A_6$ was not treated and served as control. The differently treated and untreated soy milk samples were subjected to sensory evaluation.

### 2.8 Sensory evaluation

The sensory evaluation panel consisted of 9 trained students and staff of the University of Ibadan. They were requested to rate the samples for parameters such appearance or color, taste, aroma and general acceptability. The ratings were carried out based on a nine - point Hedonic scale ranging from like very much (9 points) to dislike very much (1) as described by Larmond (1977). The results obtained were subjected to analysis of variance using one way ANOVA. Differences within the means were separated using Duncan's multiple range test according to Duncan (1955) and the two best acceptable samples were selected for further use in shelf life monitoring.

### 2.9 Treatment of soy milk samples and shelf life study

Two hundred ml of soymilk samples (A1, $A_2$ and $A_6$) were transferred differently into six presterilized 500ml screw capped Erlenmeyer flasks in duplicates. All the flasks were pasteurized for 30 min. at 60-70$^0$C in controlled water bath. A set of the flasks was refrigerated and the other set was unrefrigerated and placed on the laboratory bench. One ml was taken daily from each sample and serially diluted to ($10^{-5}$) from which 0.1ml was removed from the third ($10^{-3}$) dilution and inoculated separately on nutrient agar and plate count agar. Incubation was carried out at 37$^0$C for 24h and the observed number of colonies was counted to determine the colony forming unit (CFU/ml).

## 3. RESULTS AND DISCUSSION

Table 1 shows the Gram reaction and the biochemical characteristics of spoilage bacteria of soymilk.

A total of thirty two bacteria isolates was obtained from spoilt soy milk and identified as *Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Serratia marcescens* and *Proteus vulgaris* based on gram reaction and biochemical characteristics and with reference to Bergey's manual of systematic bacteriology. Soymilk is a popular dairy beverage commonly consumed in most part of Africa because it is a cheap source of protein. The presence of microorganisms in spoilt soymilk had been previously reported by Mbajiuka et al. (2014) and the ability of these spoilage microorganisms to survive in spoilt soymilk milk might be due to the possession of proteolytic enzymes which are capable of breaking down soymilk to simple end products such as amino acids which the microorganisms can utilize for growth and metabolism.

**Table 1** Gram reaction and biochemical characteristics of the spoilage bacteria of soy milk

| Catalase | Citrate | Gelatin hydrolysis | Gram stain staining | I ndole | Methyl red | Oxidase | Shape | Urease | Arabinose | Glucose | Inositol | Lactose | Maltose | Mannose | Mannitol | Mellibiose | Raffinose | Rhamnose | Salicin | Sorbitol | Sucrose | Starch | Xylose | Probable identify |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + | + | + | - | - | + | - | Rod | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + | *Proteus mrabilis* |
| + | + | + | - | - | - | + | Rod | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | *Pseudomonasaeruginosa* |
| + | + | + | - | - | - | - | Rod | + | - | + | - | - | + | + | + | - | - | - | + | + | + | - | - | *Serratia marcescens* |
| + | + | - | - | - | - | + | Rod | + | + | + | + | + | + | + | + | + | + | + | + | + | + | _ | + | *Klebsiella pneumonia* |
| + | + | + | - | + | + | - | Rod | + | - | + | - | - | + | - | - | - | - | - | - | - | + | - | + | *Proteus vulgaris* |

- indicates no reaction  + indicates reaction

Table 2 shows the in vitro antimicrobial activity of ethanol extract of *C. fistula* seed against spoilage bacteria. All the spoilage bacteria were susceptible to the two concentrations of the extract tested with different inhibition zones with *Pseudomonas aeruginosa*, showing the widest inhibition zone of 21.0±0.2 mm followed by *Klebsiella pneumonia* with 16.0± 0.1 mm while the least inhibition zone of 10 ± 0.1 mm was recorded by *Serratia marcescens* at 100 mg/ml concentration. At 150 mg/ml concentration the widest zone inhibition of 24.0±0.4 mm was seen in *Pseudomonas aeruginosa* followed by inhibition zone of 19.0±0.1mm demonstrated by *Klebsiella pneumoniae* while the least zone inhibition of 12 ±0.1 mm was recorded by *Serratia marcescens* The standard antibiotic used at1mg/ml concentration inhibited *Serratia marcescens, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris* and *Proteus mirabilis* showing inhibition zones of 25±0.2 mm, 33±0.3 mm, 30.0±0.4 mm, 31±0.3 mm and 30±0.3 mm respectively. In this study, it was observed that all the zones of inhibition observed were >10 mm which implies that ethanol extract of *Cassia fistula* seed demonstrated significant antimicrobial activity against isolated soymilk spoilage bacteria. According to Nand et al. (2012), all zones of inhibition measuring 10 mm and above produced by the extract against test organisms were considered effective. The presence of five phytochemicals compounds in the extract of *C. fistula* seed had been previously reported by Bhalodia et al. (2011), Subramanion et al. (2011), Kulkarni et al.(2015) and these might be responsible for the observed antimicrobial activity (Bhalodia et al., 2011; Subramanion et al., 2011; Zhang and Kin, 2008; Kulkarni et al., 2015). The mechanisms of action of plant constituents e.g. polyphenol in eliciting antimicrobial activity is achieved by inhibition of hydrolytic enzyme, activity of cell wall and cytoplasmic membrane, microbial adhesion, transport protein and non specific interactions with carbohydrate (Cowan,1997). In addition, the effectiveness of the extracts is partly dependent on the type of solvent used because the extracting solvents which are organic in nature are capable of dissolving organic compounds efficiently to release their active compounds which inhibit the growth and metabolism of bacteria(Aboaba et al., 2011; Usman et al., 2015). There are several reports that showed that alcoholic extract has the best anti-microbial activities when compared to other solvents (Neelam et al., 2012; Bameri et al., 2013; Krishnaveni, 2016). The presence of these compounds in higher amounts in the *C. fistula* seed extract explains their bioactive potentials which can be exploited in food safety and preservation.

**Table 2.** Zone of inhibition and *in vitro* antimicrobial activity of *C. fistula* ethanol seed extract against spoilage microorganisms of soymilk

| Organism | 100 (mg/ml) | 150 (mg/ml) | Ciprofloxacin (1mg/ml) |
|---|---|---|---|
| | Zone of inhibition of extract (mm) | | |
| *Serratia marcescens* | 10 ± 0.1$^{a}$ | 12 ±0.1$^{b}$ | 25±0.2$^{c}$ |
| *Pseudomonas aeruginosa* | 21.0±0.2$^{a}$ | 24.0±0.4$^{b}$ | 33±0.3$^{c}$ |
| *Klebsiella pneumonia* | 16.0± 0.1$^{a}$ | 19 0±0.1$^{b}$ | 30.0±0.4$^{c}$ |
| *Proteus vulgaris* | 14± 0.2$^{a}$ | 18± 0.1$^{b}$ | 31± 0.3$^{c}$ |
| *Proteus mirabilis* | 14±0.1$^{a}$ | 15±0.3$^{b}$ | 30± 0.3$^{c}$ |

Results are expressed as means ± S.D for triplicates. Means across the same row with different letters indicate statistically significant difference ($P \leq 0.05$).

The result of the quantitative and qualitative analyses of the phytochemicals present in ethanol seed extract of *C. fistula* is shown Table 3. The result revealed that five (5) phytochemicals compounds such as alkaloids, saponins, tannins, flavonoids and cardiac glycosides were present. The highest phytochemical compound present in the seed extract was flavanoids showing a quantity of 978±0.60mg/100g followed by tannins with 657±0.446mg/100g while the least quantity of 72±0.71mg/100g was seen in cardiac glycosides. Similar studies had earlier reported the presence of these phytochemicals compounds in the seed extract of C*assia fistula* (Bhalodia *et al.*, 2011; Subramanion *et al.*, 2011; Kulkarni *et al.*, 2015). Phenolic compounds are generally known to possess potent antimicrobial, antioxidant and anti-inflammatory properties (Gupta, 2010, Subramanion *et al.*, 2011; Rizvi *et al.*, 2009). Flavonoids also known as vitamin P or plant modifiers (Veerachari and Bopaiah, 2011) and tannins have been reported to reduce the risk of coronary disease (Janaky Ranjithkumar *et al.*, 2010) while saponins in plants have been reported to show anticarcinogenic effects (Rao and Sung, 1995).

**Table 3.** Qualitative and quantitative analyses of phytochemicals constituents of C. fistula ethanol seed extract

| Phytochemicals | Presence | Amount present (mg/100g) |
|---|---|---|
| Alkaloids | ++ | 463±64.01$^{c}$ |
| Flavonoids | +++ | 978±0.60 |
| Tannins | +++ | 657±0.44$^{d}$ |
| Saponins | + | 137±0.61$^{b}$ |
| Cardiac glycosides | + | 72±0.71$^{a}$ |

+: present; ++: present in high concentration; +++: present in highest concentration

*Results are expressed as means ± S.D for triplicates. Means in the same column with different letters indicate statistically significant difference (P< 0.05).*

The result of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of extract of *Cassia fistula* seed are presented on Table 4 and 5. It was observed that the $MIC_S$ for *S. marcescens, P. aeruginosa, K. pneumoniae, P. vulgaris and P. mirabilis* were 25.0±0.2, 12.50±0.3, 6.25±0.2, 3.12±0.1, 3.12±0.1mg/ml respectively. The result of Minimum bactericidal concentration (MBC) of the ethanol extract of *Cassia fistula* seed is shown in Table 5. It revealed that the highest MBC of 50.0±0.2mg/ml was recorded for *S. marcescens* which was followed by a MBC of 25.0±0.2mg/ml, recorded for *P. aeruginosa* and least MBC of 6.25±0.2mg/ml was recorded for both *P. vulgaris and P. mirabilis*. MIC/MBC values has been reported by many researchers as a measure of antimicrobial potential (Lakshmi and Kumar, 2012; Sen and Batra, 2012; Bameri et al., 2013; Damtie and Mekonnen, 2015). In this present study, 70% of the MIC values were lower than the MBCs values, this suggests that the extract was bacteriostatic at lower concentrations and bactericidal at higher concentrations (Rahman et al., 2011). This observed result is in conformity with the previous findings of Sen and Batra (2012). The active compounds of plant extracts confer inhibitory ability which is assessed by determining the MIC of the extract an important property which serves as guide in the therapy of bacterial infections (Aboaba et al 2011).

**Table 4.** Minimum Inhibitory Concentration of *Cassia fistula* ethanol seed extract against spoilage bacteria of soymilk (mg/ml)

| Bacteria | *Serratia marcescens* | *Pseudomonas aureginosa* | *Klebsiella pnuemoniae* | *Proteus mirabilis* | *Proteus vulgaris* |
|---|---|---|---|---|---|
| **MIC** | 25.0±0.2$^{d}$ | 12.5.0±0.3$^{b}$ | 6.25±0.2$^{a}$ | 3.12±0.1$^{d}$ | **3.12±0.1$^{d}$** |

Results are expressed as means ± S.D for triplicates. Means in the same row with different letters indicate statistically significant difference ($p \leq 0.05$)

**Table 5.** Minimum bactericidal concentration of *Cassia fistula* ethanolic seed extract against spoilage bacteria of soymilk (mg/ml)

| Bacteria | *Serratia marcescens* | *Pseudomonas aureginosa* | *Klebsiella pnuemoniae* | *Proteus mirabilis* | *Proteus vulgaris* |
|---|---|---|---|---|---|
| **MBC** | 50.0±0.2$^{d}$ | 25.0±0.2$^{b}$ | 12.5±0.1$^{a}$ | 6.25.0±0.2$^{d}$ | 6.25.0±0.2$^{d}$ |

Results are expressed as means ± S.D for triplicates. Means in the same column with different letters indicate statistically significant difference ($p \leq 0.05$)

The result of sensory evaluation of treated and untreated soymilk samples is presented in Table 6. It was observed that soymilk (A2) treated with 10% extract had the highest overall acceptability by recording 4.9±0.48, 4.5±0.71, 4.0±0.84, and 4.3±0.10 for colour, aroma, taste and general acceptability respectively. This was followed by the sample (A1) treated with 1%, and least overall acceptability recorded by the sample A5. The highest overall acceptability of soymilk treated with 10% of the extract might be due partly to flavour enhancing property of the phytochemicals present in *Cassia fistula* which is dependent on quantity to an extent.

Table 7 shows the bacteria count of treated and untreated soy milk samples during storage at refrigerated and room temperatures. It was observed that the soymilk sample supplemented 1% extract under refrigeration temperature showed bacteria growth on the 4th day of storage which increased from $1.0x10^{2}$ CFU/ml to $4.0 \times 10^{6}$ CFU/ml on the 9$^{th}$ day while bacterial growth was detected in the same sample stored at room on the 3$^{rd}$ day which increased from $1.0x10^{2}$ CFU/ml to $4.0 \times 1.0^{8}$ CFU/ml on the 9$^{th}$ day. However in the sample supplemented with 10% extract under refrigeration temperature, bacteria growth was detected on the 9$^{th}$ day of storage recording $1.0x10^{2}$ CFU/ml, while in the same sample under room temperature bacterial growth was detected on the 7$^{th}$ day which increased from $2.0x10^{2}$ CFU/ml to $3.0 \times 10^{4}$ CFU/ml on 9$^{th}$ day of storage. In the control/ untreated sample under refrigeration temperature bacteria growth was detected on the 3$^{rd}$ day of storage increasing from a bacterial count of $1.0x10^{3}$ CFU/ml to $2.0 \times 10^{9}$ CFU/ml, while in the same sample under room temperature bacterial growth was detected on the 2$^{nd}$ day which increased from $2.0x10^{2}$ CFU/ml to $9 .0 \times 10^{9}$ CFU/ml on 9$^{th}$ day of storage.

The use of natural products in the preservation of food and shelf extension is well documented, Kabiru *et al.* (2012); Kohli *et al* (2017); Oramadike and Ogunbanwo (2017). The highest preservative potential observed with the 10% supplemented extract as seen in this study could be due to the high amount of extract introduced into the soymilk when compared to the 1% extract treated sample. Shelef (1983) reported that higher amount of spices extract is very efficient in retarding microbial growth in food than culture medium. At the same time, the amount of the extract added to food should be monitored with sensory evaluation, so that this will not render the food product unacceptable to consumers.

**Table 6.** Sensory evaluation of treated and untreated samples of soymilk

| Soymilk samples | Concentration of extracts (% v/v) | Colour | Aroma | Taste | Acceptability |
|---|---|---|---|---|---|
| A1 | 1 | 4.8±0.45$^{e}$ | 4.0±0.71$^{d}$ | 3.8±0.84$^{c}$ | 4.0±1.0$^{e}$ |
| A2 | 10 | 4.9±0.48$^{e}$ | 4.5±0.710$^{c}$ | 4.0±0.48$^{c}$ | 4.3±0.10$^{e}$ |
| A3 | 20 | 3.3±0.55$^{d}$ | 3.5.0±0.0$^{d}$ | 3.6±0.45$^{c}$ | 3.5±0.55$^{c}$ |
| A4 | 30 | 3.2±0.55$^{d}$ | 3.3±0.55$^{b}$ | 3.4±0.45$^{c}$ | 3.3±0.45$^{d}$ |
| A5 | 40 | 3.0 ±0.45$^{b}$ | 3.1±0.89$^{b}$ | 3.0±0.55$^{a}$ | 3.0±0.55$^{b}$ |
| A6 (untreated) | | 4.2.4±0.89$^{a}$ | 3.6±0.84$^{a}$ | 3.8±0.55$^{b}$ | 3.8±0.84$^{a}$ |

Results are expressed as means ± S.D for triplicates. Means in the same row with different letters indicate statistically significant difference ($p \leq 0.05$).

**Table 7.** Shelf life monitoring of soymilk samples stored at refrigeration and room temperatures

| | | Days of Storage / (CFU/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Refrigerated | 1% extract | N.D | N.D | N.D | 1.0 x $10^2$ | 4.0 x $10^3$ | 2.0 x $10^4$ | 1.0 x $10^5$ | 6.1 x $10^5$ | 4.0 x $10^6$ |
| | 10% extract | N.D | N.D | N.D | N. D | N. D | N.D | N.D | N.D | 1.0 x $10^2$ |
| | Untreated (control ) | N.D | N.D | 1.0 x $10^3$ | 4.0 x $10^4$ | 2.0 x $10^5$ | 6.0 x $10^6$ | 3.0 x $10^7$ | 1.0 x $10^8$ | 2.0 x $10^9$ |
| Unrefriagerated | 1% extract | N.D | N.D | 1.0 x $10^2$ | 2.0 x $10^3$ | 3.0 x $10^4$ | 4.0 x $10^5$ | 1.0 x $10^6$ | 3.0 x $10^7$ | 4.0 x 1.0$^8$ |
| | 10% extract | N.D | N.D | N.D | N.D | N.D | N.D | 2.0 x $10^2$ | 1.0 x $10^3$ | 3.0 x $10^4$ |
| | Untreated (control) | N.D | 1.0 x $10^2$ | 4.0 x $10^3$ | 3.0 x $10^4$ | 8.0 x $10^5$ | 8.0 x $10^7$ | 5.0 x $10^8$ | 8.0 x $10^9$ | 9.0 x $10^9$ |

The factor conferring preservative ability to plant extracts and the different mechanism of action have been discussed earlier and the advantage of plant extracts over chemical preservatives is seen in their non-toxic property as they generally are regarded as safe (GRAS) and food grade. The effect of refrigeration on shelf life extension is well observed in this study as the samples in the refrigeration temperature demonstrated extended shelf life than their counterparts at room temperature. The ability of refrigeration to extend shelf life which is the mostly employed preservative method in most houses is linked to low temperature effect which inhibits survival and growth of spoilage organisms. However, combination of plant extract and refrigeration increased the shelf life of soy milk in study

## 4. CONCLUSION

This investigation confirms that the ethanol extract of *C. fistula* seed possess antimicrobial activity against spoilage bacteria of soymilk and can be applied in extending the short shelf life of the milk.

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