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RESEARCH ARTICLE

Isolation and Identification of Bacteria From Dried Fermented Leaves of *Cassia obtusifolia* .L (Kawal)

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Abstract

The present study of*Cassia obtusifolia* leaves (Kawal) were obtained from Darfur region western Sudan (Kutum, Jineena and Zalingie). The objective of this study is to isolate bacteria from different samples of dried fermented *cassia obtusifolia* leaves (kawal) and to identify bacteria by using different biochemical tests,which is present in natural environment and fermented traditionally as survival, the study was carried out in Khartoum state-Sudan , during February 2019 to September 2019, the samples were collected from three area (Kutum, Jineena and Zalingie) . The traditional method for fermentation was used and the bacteria were isolated by using nutrient agar. The biochemical tests were used to determine type of bacteria and the results were cited positive with catalase, citrate, urease, motility tests, while gas and indol were showed negative result, the microorganisms were examined ,.The sugar fermentation tests for bacteria were recorded positive with glucose, sucrose, mannitol tests, while it showed negative at lactose sugar . The results revealed that all the samples were positive in nutrient agar media and the isolation of microorganisms were showed that ,the*bacillusubtilis* bacteria is only microorganism that was determined in the fermented kawalleave.

Keywords: : Isolation, Darfur region, kawal, fermentation, Cassia obtusifolia.

1 | INTRODUCTION

Cassia obtusifolia is a number of (Leguminaseae Family) it is a wild spread African plant, found in waste lands in the rainy season, the leaves of the species can be fermented to (kawal) and is used by people from the eastern part of Chad and the western part of Sudan as meat replacer or meat extender[1].One method of food transformation and preservation is fermentation process that improves nutritive value, safety, hygienic, organoleptic quality of the food and detoxification of antinutrient factors[2][3][4][5][8].In Africa, the art of fermentation is widespread including the processing of fermented cereals, legumes, the fermented cereals are dominated by Lactic Acid Bacteria (LAB)

associated with yeast ,*Bacillus* species one of the microorganisms that dominates, with their major proteolytic activity to hydrolyze proteins into amino acids and ammonia[6][16]. it play an important role in providing food security, improving the nutrition and social well-being of millions of people around the world [7].They also play a very important role in the socioeconomics of developing countries. The process generally produced from plant or animal-based raw materials in combination with fungi or bacteria, which are either present in the natural environment, or added intentionally by human to obtain the desirable end-products [8].

1.1-Objective:

-The main objective of the present study is to isolate bacteria from different samples of dried fermented of *cassia obtusifolia*leaves(kawal) and to identify bacteria by using different biochemical tests.

2 | MATERIALS AND METHODS

2.1-Study area and collection of sample:

This study was carried out in Darfur region , western Sudan, it covers an area of 493,180 square kilometers (190,420 sq mi), It is largely an arid plate with the Marrah Mountains (Jebel Marra), a range of volcanic peaks rising up to 3,042 meters (9,980 ft) , with rainfall down by up to 30% over 40 years and the Sahara advancing by well over a mile every year, the soil is composing of clay plains in some areas and low hills of sandy known as algoz and sand stone hills in eastern areas. Dried fermented samples (kawal) were collected from three different places in Darfur region (Kutum, Jineina, Zalingie) from February 2019 to April 2019. All dried powdered samples were transferred to laboratory for microbial analysis.

Supplementary information The online version of this article ( ) contains supplementary material, which is avail-able to authorized users.

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2.2-Preparation of medium:

28g was suspended in 100ml of distilled water in conical flask after mixing the solution, it was heated to boiling gently to dissolve the medium completely and sterilized by autoclaving at 121ºC for 15 minutes. The medium was allowed to cool down to 45-50 ºC, and then poured in sterile Petri dishes.

2.3-Isolation of bacteria:

5g of powderedkawal samples were dissolved in 95ml sterilized distilled Water, 1ml of sample was serially diluted up to [10]^(-5) dilution. 0.1ml from each dilution was cultured by using streaking method in the nutrient agar medium ,the plates incubated at 37 ºC for 24 hours. Discrete colonies exhibited smooth, white-creamy color on nutrient agar was sub cultured on new nutrient agar plates to obtain pure cultures. The pure colonies were cultured on nutrient agar in test tubes for further identification by biochemical tests. [9].

2.4-Identification of bacteria:

All suspected isolates of bacteria were subjected to standard biochemical tests for bacterial identification by following the criteria described in {Bergey's Manual of systematic Bacteriology[10]biochemical tests include: (Indole test, urease test, motility test, citrate test, H_2S gas production test, catalase test. Followed by fermentation of (Glucose, Sucrose, Lactose, Mannitol) in addition to gram staining.[10].

2.5-Biochemical tests:

2.5.1-Gram staining:

Drop of sterile normal saline was placed in sterilized dried microscopic slide under aseptic condition. Pure colony of isolated bacteria of overnight culture was emulsified on drop of normal saline and rolled by sterile loop until thin layer of smear was prepared and allowed to dry at room temperature and fixed by passing the smeared slide rapidly over the flame. After cooling it was covered with crystal violet solution and allowed for one minute and discarded and was washed off with water, then the smear was covered with fresh iodine solution and left for one minute and washed with water, then it was rinsed with absolute alcohol for(15-20 seconds) until remove color appears. And washed with water and covered with Sufranine solution for one minute, then rinsed with

distilled water and air dried at room temperature and examined microscopically under oil emersion lens, a light microscope at X100. (the isolates were gram positive rods). The gram staining technique was used to categorize the isolates into gram negative and gram positive (Cappuccino & Sherman, 2010)[11].

2.5.2-Indole test:

Peptone water medium was prepared according to the manufacturing instructions (Micro master limited, INDIA) and distributed in test tubes ,autoclaved at 121 ºC for 15minutes and allowed to cool. Full of suspected bacterial colonies were cultured into the medium and incubated at 37 ºC for 24 hours. Five to six drops of kovac's reagent were added carefully to the culture through the internal surface of the tube. The change in the color of kovac's reagent from brown to cherry red was considered positive indole production by the organism. (the isolated bacteria were indol negative).[12].

2.5.3-Urease test:

The basal medium was prepared according to the manufacturing instructions (Scharlau) without glucose and urea, sterilized by autoclaving at 121 ºC for 15 minutes, cooled to about 50 ºC, then sterilized solution of 20%, 10% urea and glucose respectively were added. The medium was distribute in sterile test tubes and allowed to cool and solidify at room temperature in slant form. Suspected bacterial colonies were cultured in sterile loop into the medium by streaking the slant and incubated at 37 ºC for 24 hours. Positive cultures produce an alkaline reaction in the medium showing of change in color. No change in color of the medium indicates urease negative. (the isolated bacteria were urease positive)[13].

2.5.4-Motility test:

Motility test medium was prepared according to the manufacturing instructions (Hi media laboratories limited, INDIA) and distributed in test tubes, sterilized by autoclaving and allowed to cool then inoculated With suspected bacterial by stabbing through the center of the medium with sterile straight needle to approximately one-half the depth of the medium, incubated at 37 ºC for 24 hours. Motile organism migrates throughout the medium, which becomes turbid. Non-motile confined to the stab inoculums. (the isolated bacteria were motile organisms [14].

2.5.5-Citrate utilization test:

Simmon's citrate agar medium was used for citrate test. The medium was prepared according to the manufacturing instructions (Scharlau) and distributed in test tubes, sterilized by an autoclave at 121 ºC for 15 minutes, then all tubes were slanted, and then allowed to cool and solidify at room temperature. Under aseptic condition pure suspected bacterial colonies were streaked on slanted tubes by sterile loop and incubated at 37 ºC for 24 hours. The utilization of the citrate was showed by blue color; whereas non-utilization did not change (the isolated bacteria were citrate positive)[11].

2.5.6-Hydrogen Sulphide gas production test:

Kliger iron agar (KIA) medium was used for H_2S gas production test, the media was prepared according to the manufacturing instructions (Hi media laboratories limited, INDIA) and distributed in test tubes, sterilized by an autoclave at 121 ºC for 15 minutes, then all tubes were slanted, and allowed to cool and solidify at room temperature. Under aseptic condition, tubes of KIA slants were inoculated with suspected bacterial colonies by stabbing the butt and streaking the slant with sterile straight needle and incubated at 37 ºC for 24 hours. The tubes were examined for the production ofH_2S (blacking the medium by the organism). (The isolates were gas negative)[15].

2.5.7-Catalase test:

Under aseptic condition, drops of H_2 O_2 solution were placed in dried slide; pure colony of overnight culture of isolated bacteria was emulsified on the drops of H_2 O_2 solution and rolled by sterile loop, the enzyme showed by immediate production of gas bubbles. The absent of Catalase enzyme showed by no bubbles (the isolates were catalase positive). ([11].

2.5.8-Fermentation of sugars:

Sugars were prepared according to the methods described by {Bergey's Manual of systematic Bacteriology [10] with some modifications. 0.25g from each sugar was taken and added to 0.5g pancreatic digest, 0.25g NaCl, 0.0009g phenol red and dissolved in 50ml distilled water. Distributed in test tubes containing Durham tubes and closed well with

sterilized cotton and foil, and then sterilized by an autoclave at 121 ºC for 15 minutes and allowed to cool. Under aseptic condition, tubes were inoculated with suspected bacterial colonies and incubated at 37 ºC for three days. (The isolates were lactose negative and glucose, sucrose, mannitol positive) [10].

3 | RESULTS

3.1-Isolation of bacteria by nutrient agar:

All the samples collected from different places were found to be positive growth in nutrient agar.

3.2-Identification of bacteria:

In gram staining techniques the isolates were found gram positive rod Shapes of *bacilli*, some of them have rod shapes in pairs with rounded ends and some have rod shapes in chains with squire ends. In biochemical tests the isolates were found positive Catalase, Citrate, Urease and Motility, while Gas and Indol were found negative. In sugar fermentation tests the isolates were negative at Lactose, while were Positive at Glucose, Sucrose and Mannitol.

4 | DISCUSSION

3.3-Isolation and Identification of bacteria:

This study shown prevalence of bacteria in dried fermented kawal by using nutrient agar medium, the results revealed all samples were positive growth in nutrient agar medium ,these results are similar to findings reported by (AbakarIdrissLawanen, *et al*, (2016) and Dirar, *etal*. (1984) but they have used De Man Rogosa and Sharp (MRS) agar medium.The positive rod shapes were *bacillus* and identified by positive tests of catalase, citrate, urease, motility and positive fermentation of glucose, sucrose, mannitol this is similar to (Lutz *et al*, 2006) and the results which were correspond to the study were conducted by Dirar*et al* (1985)was showing the *bacillus* species as dominant bacteria in kawal samples.The only species of *bacillus* was confirmed to be *b.subtilis*that it is the catalase positive and lactose fermentation negative. The similar findings were also reported by (AbakarIdrissLawanen, *et al*, 2016). The results show that the *b.subtilis*bacterium shows prevalence in different dried fermented kawalthat found in different regions of Darfur. The same findings were also reported by (Dirar, 1984).The isolation of bacterium *bacillus* subtilis from dried fermented kawalby nutrient agar was foundpositive. Further work is requiring using molecular tool with enough samples to confirm identification of *b.subtilis*.

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