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Analysis of Phytochemical Potentiality and In Vitro Antimicrobial Properties of Jute Leaf Extracts

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Abstract
Jute leaf is used as an herb in Middle Eastern and African countries which has large number of biomolecules that show various pharmacological activities. It has been studied for antibacterial activity by disk diffusion method where it was clear that it has antibacterial activity against all the test organisms. The boiling time has effect on antibacterial activity. In case of Minimal Inhibitory Concentrations (MIC), Minimal Bactericidal Concentrations (MBC) and phytochemical activities in jute leaf liquor, it inhibits the growth of *Bacillus cereus* where the MIC and MBC was 128mg and 256mg, respectively; and Cardial Glycosides phytochemical was found in all the three varieties O-72, O-9897 and CVL-1 jute leaf liquor.

Keywords: Zone of inhibition; Minimal Inhibitory Concentrations; Minimal Bactericidal Concentrations; Phytochemical

Introduction
The jute plant is an economically important fiber crop which is widely distributed in the tropics of both the hemisphere (Islam, 2013). Jute leaves are favorite vegetables in Bangladesh. Jute leaves are well-known in our nation as a traditional cure for treating fever, getting rid of hookworm, etc. Jute is also grown in Bangladesh and other nations as a herbal medicinal plant (Balkrishna, 2008). Numerous research studies have uncovered the antibacterial quality of jute leaves, which has been believed by many country residents to possess anti-fever properties. A water extract has successfully been shown to prevent the test bacterium from multiplying. Leaf extract is frequently used in Ayurveda as an adjuvant, perhaps combining with other treatments for better results, in addition to its solitary use as a medicinal (Rahman et al., 2022; Ahmed and Sarkar, 2022). Jute is traditionally used for fiber but it has medicinal applications. From the ancient time jute leaf was used in traditional medicine, but without knowing its actual curative source (Sadat et al., 2017). It was previously discovered that the leaves of jute plants contained bioactive compounds, which may be connected with phytochemicals and have anti-bacterial properties (Rahman et al., 2022). Jute plant leaves must be used in the right amounts if they are to be effective antimicrobials. The use of biocides at sub-lethal concentrations is not only useless but can also be detrimental because low dosages are associated with a rise in bacterial tolerance to these substances, resistance to antibiotics, as well as a greater ability to create biofilm. This makes it crucial to comprehend the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of jute plant leaves. The increase in resistance to antibiotics over the last few decades has become a source of concern worldwide. Although various strategies are being devised to prevent and control this problem, bacterial resistance is becoming ever more frequent, both in clinical strains and in those found in the environment or in foodstuffs. Phytochemicals are known to work as immune-modulators and may have anti-inflammatory, anticancer and antimicrobial activities. All of these capacities are due to the phytochemicals' strong antioxidant defenses against harmful free radicals produced by the body (Rahman, 2007).

Materials and methods

Plant material and sample preparation

Jute leaf of three varieties O-72, O-9897 and CVL-1 were collected from Manikganj area of Dhaka, Bangladesh. Jute leaves liquor was prepared (Rahman et al., 2022). All the reagents and media used in this research were reagent grade and bacterial samples were ATCC standard. To get rid of dirt, the leaves were first washed in tap water and then in double-distilled water. To get rid of the dirt and other impurities, leaves were washed in distilled water. The leaves were then dried for seven days at room temperature (25°C). The dried leaves were then ground using a standard grinder, sieved through a screen, and then finely powdered for use in MIC and MBC as well as phytochemical analysis. In order to prepare the sample needed to measure the antioxidant activity, this dried powdered material was also employed in solvent extraction. The powder (100g) was mixed with 500ml methanol:water (7:3) using a shaker for 15h; then the mixture was centrifuged at 2850×g and the supernatant was decanted. The pellet was mixed again with 500ml methanol-water and the entire process was repeated once again, i.e. the extraction procedure was done twice. In a flask with a circular bottom, the supernatants from the two phases were combined, and they were then condensed under reduced pressure in a rotating evaporator. The residue was used to assess the antioxidant strength of jute.

Preparation of bacterial suspension and Analysis of shelf life

The bacterial samples of *Pseudomonas* sp., E. *coli*, *Klebsiella* sp., S. *aureus*, M. *luteus and* B. *cereus* were used in present study. Standard loop full suspension of the test organisms was aseptically streaked onto nutrient agar slants and was incubated at 37°C for 24 hours. The bacterial growth was then harvested from the appropriate slant and sterile 1 ml of normal saline (0.85 gm NaCl in 100 ml of distilled water) was used to create a suspension. The suspensions were stored in the refrigerator at 4 °C until they were needed. In the antimicrobial experiment, Kanamycin was used as the positive control (P), and water as the negative control (N). The jute leaf liquor was made using 10% dried leaves and was boiled for 5, 10, and 15 minutes. To test the antibacterial activity, *S. typhi* were infected on nutrient agar media using the spread plate method, and liquor was then added to the middle of the bacterial inoculation plate.

Determination MIC and MBC

The MIC of the antimicrobials was determined by the method involving micro dilution in culture broth, as indicated by the clinical and laboratory standards institute of the United States of America. In this process, different concentrations of antimicrobials (jute leaf tea liquor) were used. Three replicates were performed for each strain and antimicrobial compound. Five colonies of each strain were taken from the Muller Hinton agar (MHA) plates, inoculated into 9mL of Muller Hinton Broth (MHB), and incubated at 37°C for 18 to 24h. In this experimental work, polystyrene micro-titer plates with one hundred wells were used. The wells were filled with a total volume of 200µL, made up of 20µL of the antimicrobial solution at a range of concentrations and 180µL of the third dilution of the inoculums to obtain a final concentration in the well of approximately 10^5 cfu/mL. The concentration of the inoculums was confirmed by plating. Negative controls with 200µL of MHB and 200µL of the antimicrobial solutions and positive controls with 200µL of the bacterial inoculums were used. Growth was determined by measuring the optical density of each sample in the range 480 to 520nm. The value for MIC was set as the minimum concentration of the antimicrobial substance necessary to prevent bacterial growth after 48h of incubation at 37°C (Rodríguez-Melcón et al., 2021). The growth limit was deemed to be a value of 0.200 for $OD_{480–520}$. Strains were classified as susceptible, resistant, or with reduced susceptibility (intermediate) based on established criteria.

Muller Hinton Broth (102.4µl) + Initial leaf extraction concentration (102.4µl)
=> Final concentration (ml/ml) (51.2ml)

The dilution in broth method was used to calculate the MBC for the antimicrobials (jute leaf tea liquor). A volume of 0.1mL was removed from the wells in the micro-titer plates where no growth was observed after 48h of incubation at 37°C, and was then inoculated onto the surface of MHA

plates. The material concentration at which no colonies formed under the incubation conditions for 48 hours at 37°C was chosen as MBC. Since the limit of detection for this technique is 10 cfu/mL, the absence of any growth on a MHA plate indicated that the concentration lay below this value. The initial concentration of 10^5cfu/mL had thus been reduced to below 10cfu/mL. Consequently, the MBC was effectively deemed to be the minimum concentration of antimicrobial capable of inactivating more than 99.99% of the bacteria present. Three replicates were performed for each strain and antimicrobial compound (Parvekar et al., 2020; Rodríguez-Melcón et al., 2021).

Qualitative tests (Phytochemical analysis)

The qualitative tests for phytochemicals were performed according to several previously published standard protocols. 10g of powdered material was mixed in 100ml of double distilled water and the mixture was placed in magnetic stirrer for 10h. The mixture was filtered through Whatman filter paper No. 1 and the filtrate was used for the following phytochemical tests (Tiwari et al., 2011).

Test for Tannin (Lead acetate test)

The presence of a reddish precipitate, an indication of tannin, was sought after the addition of 2ml of test extract to 1% lead acetate. A few drops of the 0.1% ferric chloride solution were added to 10ml of the aqueous extract. When a brownish-green or blue-black precipitation occurred, tannin was present.

Test for Diterpenes (Copper acetate test)

After being diluted in distilled water, test extract was treated with 8–10 drops of copper acetate solution. The production of emerald green color denotes the existence of diterpenes.

Test for Anthocyanin

The pink red color changed to a blue violet hue when 2ml of the aqueous test extract was added to 2ml of the 2N HCl and NH_3, which was taken as evidence that anthocyanin was present.

Test for Coumarin

A yellow color was produced when 3ml of 10% NaOH was applied to 2ml of an aqueous test extract, indicating the presence of coumarin.

Test for Leucoanthocyanin

5ml of iso-amyl alcohol were added to 5ml of the aqueous test extract. Leucoanthocyanin is present when the upper layer appears red, which indicates its presence.

Test for Phytosterol (Salkowskis test)

The test extract was treated with chloroform and filtered after that. The filtrate was then mixed thoroughly, and a few drops of concentrated H_2SO_4 were added. The test was successful when a rich red hue developed.

Test for Phenol (Ferric Chloride test)

When the test extract is treated with 4–5 drops of an alcoholic $FeCl_3$ solution, a bluish black hue results, signaling the presence of phenol.

Test for Phlobatannins

It is considered as evidence that phlobatannins are present when red ppt forms after heating aqueous extract from the sample with 1% aqueous HCl. 2ml of strong HCl was added to 10ml of aqueous extract, which was then heated for two minutes. The presence of phlobatannins was determined by the development of a red precipitate.

Test for Cardial Glycosides (Keller-Killani Test)

A drop of $FeCl_3$ and two milliliters of glacial acetic acid were added to the extract during the treatment process. A brown color ring that forms is a sign of cardial glycosides. 5 ml of methanolic extracts and 2 ml of glacial acetic acid were added to a 2% ferric chloride solution. The test tube's

wall was then lightly coated with 1 ml of concentrated H_2SO_4. A brown ring appeared at the boundary between the two liquids, indicating the presence of glycosides.

Test for Flavonoid (Zinc dust test)

A successful test was achieved when 2 ml of the test extract were treated with Zn dust and a few drops of strong HCl. 2g of powdered material and 10 ml of ethyl acetate were mixed together, then heated in a water bath for five minutes. After cooling, the solution was filtered, and the filtrate was mixed with 4 ml of liquid ammonia solution (10%) before being vigorously shaken. The yellow coloring, which looked to be brought on by flavonoids, confirmed the existence of phenolic chemicals.

Test for anthraquinone

A magnetic stirrer was used to mix 20 ml of benzene and 0.5 g of powder for 4 h at room temperature. Following filtration, the mixture was combined with 0.5 ml of a 10% ammonia solution and violently shaken with 10ml of the filtrate. Because of the violet hue, anthraquinones were present at the layer phase.

Test for Saponin (Foam test)

5ml of the test extract and 20ml of distilled water were combined, then swirled for 15 minutes in a graduated cylinder. Saponin evidence is considered to be the creation of foam. 0.5g of the powdered material was added to 15ml of double-distilled water, which was then vigorously shaken. Saponin was present as shown by the vigorous and long-lasting foaming that developed.

Results and discussion

Among the bacteria tested for antibacterial activity, jute leaf tea liquor showed potential activity against different bacteria shown in Fig. 1 and Table 1, where it was found that S. *aureus* and M. *luteus* zone of inhibition and other test bacterial samples. The clear zone formed in the plate where inoculated bacteria could not grow. Bacterial growth was inhibited due to liquor of jute leaf. This is a clear illustration of the antibacterial effects of jute leaf liquor. It is claimed that several volatile compounds are effective antimicrobial agents. Additionally, because of the plant itself or the compounds it contains, its protective characteristic prevents germs from spoiling the food it is used in or increasing its shelf life. Both its flavor and health-promoting properties may be to blame for its widespread consumption today as a plant supplement to other foods and for human consumption. To determine the synthesis of metabolites, other antibacterial properties, and the formation of novel chemicals in this jute leaf, more investigation is obviously required.

Among the bacteria tested for antibacterial activity, jute leaf liquor showed potential activity against S. *typhi* where the clear zone formed in the plate where inoculated bacteria could not grow. This was a clear indication of antibacterial property of jute leaf. The liquor was prepared with different boiling time e.g. 5, 10 and 15 minutes which has an effect on inhibition, i.e. the longer boiling showed more inhibition (Fig. 1). It has been reported that many volatile components are effective against Gram-positive and Gram-negative bacteria. The leaves of plant are used commonly by the people in food as a vegetable. The human body is able to directly absorb the different compounds and due to the low concentration of these compounds, it has transpired that the plant particularly provides effective protection against infection. The food it is used in also benefits from this protective characteristic, which prevents germs from spoiling it through the plant itself or the substances it contains (Gokhale and Kokate, 2008). Both its flavor and health-promoting properties may be to blame for its widespread consumption today as a plant supplement to other foods and for human consumption. To determine the synthesis of metabolites, other antibacterial properties, and the formation of novel chemicals in this jute leaf, more investigation is obviously required.

Determination of MIC and MBC

The MIC and MBC of the antimicrobials were determined by the method involving micro-dilution (polystyrene micro-titer plates with one hundred wells) in culture broth where different concentrations of antimicrobials (jute leaf tea liquor) were used. Five colonies of each strain were

taken from the Muller Hinton agar (MHA) plates, inoculated into 9mL of Muller Hinton Broth (MHB), and incubated at 37°C for 18 to 24h (Fig. 2, Table 2). The MIC and MBC values for B. *cereus* were found to be 128 mg and 256 mg, respectively.

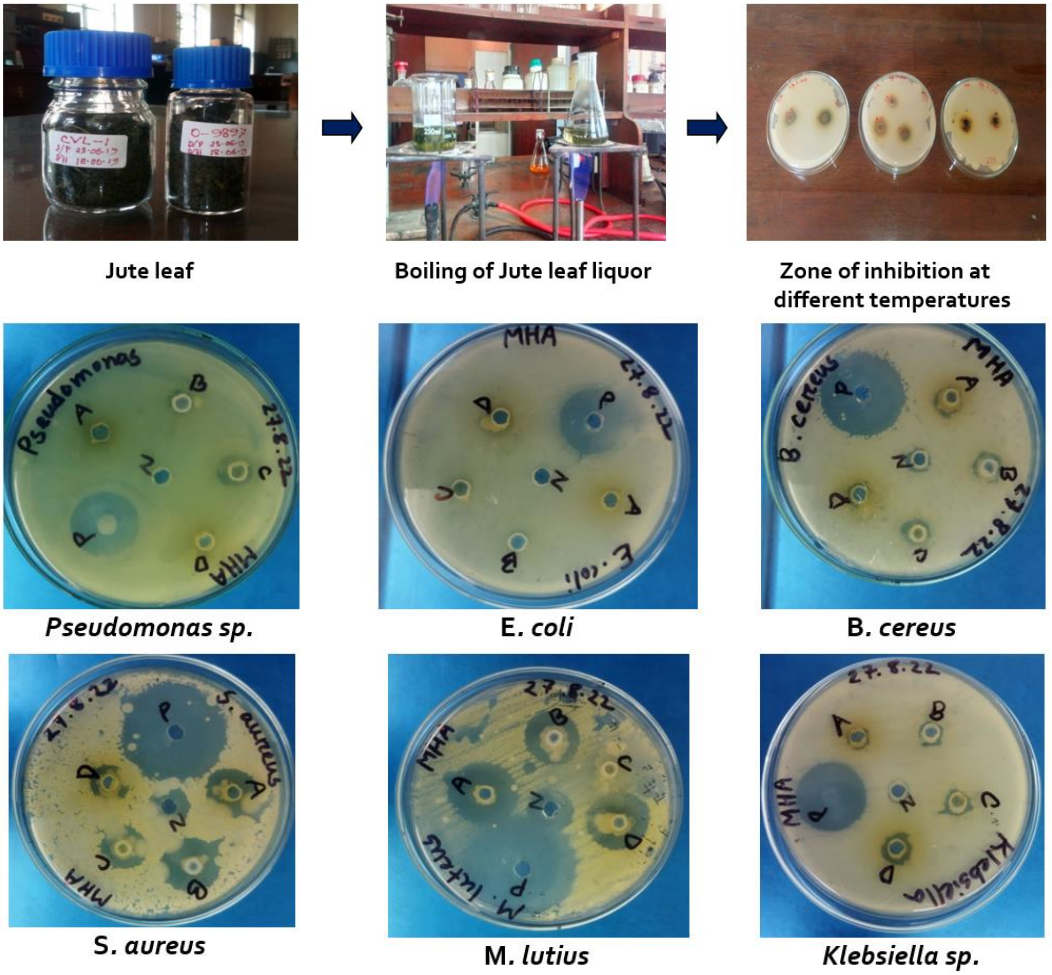


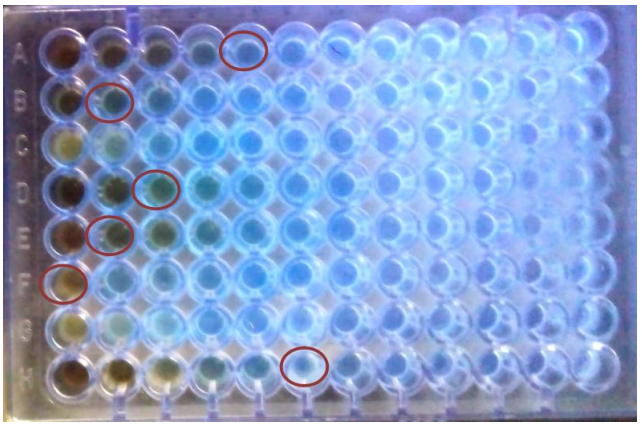
Fig. 1: Jute leaf tea and zone of inhibition of inoculation of bacteria

Table 1: Zone of inhibition (mm) of bacteria of water extraction jute leaf liquor.

Sample	*Pseudomonas* sp.	E. *coli*	*Klebsiella* sp.	B. *cereus*	M. *luteus*	S. *aureus*
o-72	-	-	-	10	12	contamination
o-9897	11	-	-	11	13	contamination
CVL-1	-	-	-	12	14	contamination
Positive Control (P) (Kanamycin)	22	23	14	18	24	30
Negative Control (N)	-		-	-	-	8

Qualitative estimation of phytochemicals

The phytochemical screening carried on the leaf extract of jute revealed the presence of some active ingredients such as alkaloids, cardiac glycosides, saponins, phenols and flavonoids. These phytochemicals are known to possess therapeutic activity which justifies its uses as traditional medicine. In a previous report, it has been documented that these phyto-constituents may be responsible for several pharmacological activities like wound healing, cholesterol lowering and antidiabetic activity.



Sample = 2.5g
Solvent (D.H_2O) = 22.5ml
A-D = S. *aureus*
E-H = M. *luteus*

Fig. 2: MIC and MBC of the antimicrobials by the method involving micro dilution

Table 2: MIC and MBC of Jute leaf extraction

Bacterial Sample	Sample of leaf extraction		Replicate Final concentration (ml)											
			1	2	3	4	5	6	7	8	9	10	11	12
			512	256	128	64	32	16	8	4	2	1	PC	NC
S. aureus	o-9887	Broth dilution	-	-	-	-	+	+	+	+	+	+	+	-
		Growth on plate	-	-	+	+	+	+	+	+	+	+	+	-
M. luteus		Broth dilution	-	-	+	+	+	+	+	+	+	+	+	-
		Growth on plate	-	+	+	+	+	+	+	+	+	+	+	-
S. aureus	o-72	Broth dilution	-	-	+	+	+	+	+	+	+	+	+	-
		Growth on plate	-	+	+	+	+	+	+	+	+	+	+	-
M. luteus		Broth dilution	-	+	+	+	+	+	+	+	+	+	+	-
		Growth on plate	+	+	+	+	+	+	+	+	+	+	+	-
B. cereus	CVL-1	Broth dilution	-	-	-	+	+	+	+	+	+	+	+	-
		Growth on plate	-	-	+	+	+	+	+	+	+	+	+	-
M. luteus		Broth dilution	-	-	-	-	-	-	+	+	+	+	+	-
		Growth on plate	-	-	-	-	+	+	+	+	+	+	+	-

PC = Positive control (Kanamycin), No Bacterial suspension; NC = Negative control, with Bacterial suspension (100 µl).

It has been known that plant steroid, flavonoids and phenols are antioxidants (Raaman, 2015). Antioxidant values may be dependent on the presence of different phytochemicals such as phenols alkaloids, flavonoids, saponins, tannins etc (Harborne and Willians, 2000). The phenolic

compounds are responsible for the variation in the antioxidant activity of the plant. It has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as alkaloids, flavonoids, phenols and tannins. Antioxidants molecules of plants generally belong to secondary metabolic products. These molecules protect cells against the destructive effects of reactive oxygen species (ROS), such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^\bullet$) formed by the partial reduction of oxygen (Roy et al., 2013; Shrmila et al., 2007). Cellular ROS are generated endogenously as a normal metabolic function in the mitochondrial oxidative phosphorylation, and or they may occur due to interactions of the cell with exogenous sources such as xenobiotic compounds. An imbalance between antioxidants and ROS results in oxidative stress in cell and leading to cellular spoil. This situation often linked to physiological disorders viz., cancer, ageing, atherosclerosis, inflammation, ischemic injury and neural degeneration (Ogundipe et al., 2001; Islam, 2012).

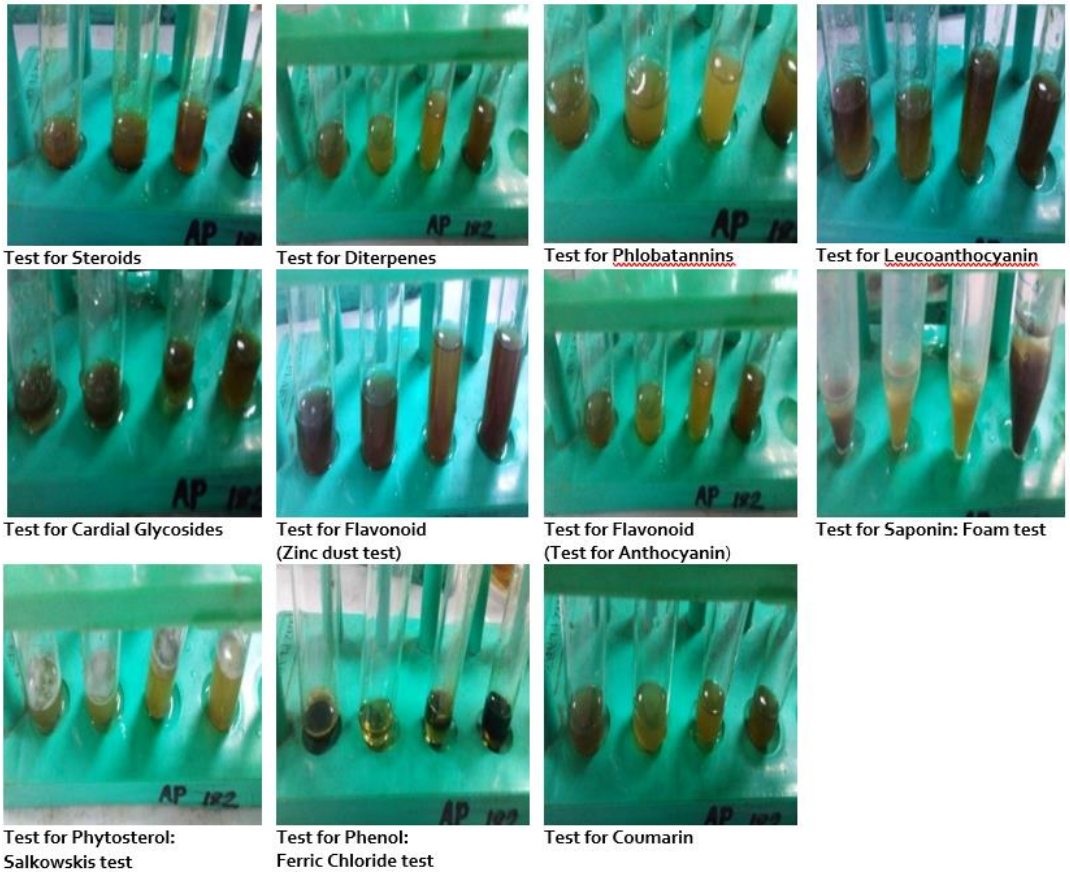


Fig. 3: Qualitative estimation of phytochemicals in jute leaf tea liquor

Table 3: Phytochemicals in different varieties of jute leaf tea liquor

Water Extract	0-72	0-9897	CVL-1
Test for Steroids	-	-	+/-
Test for Diterpenes	-	-	-
Test for Phlobatannins	-	-	-
Test for Tannin Lead acetate test	-	-	-
Test for Cardial Glycosides	+	+	+
Test for Flavonoid: Zinc dust test	+	-	+
Test for Anthocyanin	-	-	-
Test for Phytosterol: Salkowskis test	-	-	-
Test for Phenol: Ferric Chloride test	-	+	+
Test for Coumarin	-	-	-
Test for Leucoanthocyanin	-	-	-
Test for Saponin: Foam test	-	-	-

Conclusion

Jute leaf tea liquor inhibits growth of S. *typhi* where the MIC and MBC was 128mg and 256mg, respectively. An awareness of the MIC and MBC for jute leaf tea are necessary to destroy these test organisms which may assist with choosing the most effective dose of jute leaf tea liquor for controlling these microorganisms (Rodríguez-Melcón et al., 2021), whether in the food industry or in the health system. It was established that the deadly concentrations of the jute leaf liquor were far lower than the usual dosages. It is clear that the necessary controls for these bacteria must be put in place given the high occurrence of resistance to the majority of the tested antibiotics. Moreover, Jute leaf tea liquor has some phytochemical properties where Cardial Glycosides was found in all the three varieties O-72, O-9897 and CVL-1 jute leaf liquor. Present investigation concludes that leaves are the rich source of various phytochemicals. Qualitatively and quantitatively aqueous extract yields more phytochemicals than the other extracts. All the extracts were shown antibacterial properties but comparatively the butanolic, acetone and methanolic extract were found more effective. Thus, the leaves extract of may be used as a source of antibiotics. Dietary supplementation through natural antioxidants in place of synthetic antioxidants is necessary for strengthening the antioxidant system of the body by inhibiting free radical generation and thus preventing chronic diseases. Natural antioxidants have drawn a lot of attention because it is thought that they are secure sources of natural goods. Further research must be done to determine whether this alcoholic beverage is effective for pharmaceutical reasons.

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ZA conceived the concept, wrote and approved the manuscript.

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Competing interest
The author declares no competing interests.

Ethics approval
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