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ANTIMICROBIAL STUDY OF KUTAJADI CHURNA AND KUTAJADI SUSPENSION: AN IN-VITRO STUDY

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ABSTRACT

Introduction: Diarrhoea affects 3-5 billion people globally each year, causing 5 million deaths especially children under 5 years. The major causative agents of diarrhea include a variety of enteric pathogenic bacteria. Kutajadi churna is an unexplored polyherbal ayurvedic formulation which is indicated for raktatisara (bloody diarrhea). Kutajadi suspension is the modified form of kutajadi churna aiming better ease of administration, longer shelf life and bioavailability. **Aim:** To study the antimicrobial activity of Kutajadi churna and Kutajadi suspension. **Methodology:** Agar well diffusion and Broth dilution method were carried out as per the standards for testing. **Result:** Kutajadi churna shown presence metabolites like tannins, saponins, flavanoids etc which are responsible for antimicrobial, antidiarrheal, antioxidant activity. Kutajadi churna shown no growth for *E. coli* (2 lac ppm), *S. typhi* (2 lac and 1 lac ppm), *S. flexineri* (2 lac and 1 lac ppm), *S. aureus* (2 lac,1 lac and 50,000 ppm). Kutajadi suspension shown no growth for *E. coli*, *S. typhi*, *S. flexineri* and *S. aureus* (20 lac ppm). **Conclusion:** The Kutajadi churna and Kutajadi suspension possess antimicrobial activity against *E. coli*, *S. typhi*, *S. flexineri*, and *S. aureus* at various concentrations.

KEYWORDS: antimicrobial, MIC, churna, suspension, kutajadi, microorganism.

INTRODUCTION

Diarrhoea affects 3-5 billion people globally each year, causing 5 million deaths, according to the World Health Organization (WHO).[1] Children, on the other hand, are more vulnerable to the condition, which is one of the major causes of death in children under the age of five.[2] There is an increase in faeces flow rate, with or without blood and mucus, as well as increased secretion and decreased fluid absorption, resulting in water and electrolyte loss.[3] The major causative agents of diarrhoea include a variety of enteric pathogenic bacteria such as *Salmonella typhi*, *Shigella flexneri*, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae*, and *Candida albicans*.[4] Antibiotic resistance is on the rise all across the world.[5] Antimicrobial agents can be found in abundance in traditionally used herbs and medications.[6]

Kutajadi churna is an unexplored polyherbal ayurvedic formulation which is indicated for raktatisara (bloody diarrhoea).[7] It comprises of combination of five antidiarrheal drugs namely *Holarrhena antidycentrica* Wall. (*Kutaja*), *Cissampelos pareira* Linn. *(Patha)*, *Zingiber officinale* Rosc. *(Shunti)*, *Aegel marmelos* Corr. (*Bilwa*) and *Woodfordia fruiticosa* Kurz. *(Dhataki*) with honey as anupana. Due to certain demerits of churna like hygroscopicity, difficulty in swallowing for pediatric and geriatric group etc, the *Kutajadi Churna* (Solid) was developed into Kutajadi Suspension (Liquid) form for its better utility and its antimicrobial activity was evaluated.

MATERIAL AND METHODS

Materials

Conical flasks, Petri plates, Micropipette, Test tubes, pH Meter and paper, Sterile cotton swab, Biosafety cabinet II, Bacteriological Incubator (37°C).

Medias required

Mueller Hinton Broth (MHB), Mueller Hinton Agar (MHA), Standard Drug (Streptomycin 15,000 ppm), Solvent control (Methanol).

Test organism

Gram positive and gram-negative microorganism *like Staphylococcus aureus* (NCIM 2127), *Escherichia coli*

(NCIM 2256), *Salmonella typhimurium* (NCIM 2501), *Shigella flexneri* (ATCC 9199) responsible for diarrhea were selected for the test from certified laboratory.

Test sample

Both Kutajadi churna and Kutajadi suspension was tested for antimicrobial activity against above said microorganisms.

Agar Well Diffusion Method

Inoculum preparation: Transfer a loopful of bacterial culture from working stock slants to 5ml of MHB and incubated at 37ºC till getting a visible turbidity equivalent to 0.5 MacFarland unit). Prepare MHA plates in sterile petridish. Allow to solidify. Take a sterile swab and dip it into the broth culture. Gently squeeze the swab against the inside of the tube to remove excess fluid in the swab. Use the swab with the test organism to swab plate for a lawn of growth. After the swabbing, allow the plate to dry for 5 minutes. Using sterile Cork Borer of 8mm diameter prepare wells on the swabbed agar plates. 100µl sample and required concentrations were added to the well using micropipette. Antibiotic control was also kept. If the sample is prepared using any diluent/solvent, it should be also kept as a vehicle control. Kept the plates in the biosafety cabinet till the diffusion of sample occurs and after that incubate the bacterial plates at 37°C 24 hours. After incubation, using a ruler measure the diameter (mm) of the zone of inhibition. Record the results.

MIC Determination-Macro Broth Dilution Method

Arrange the tubes in a test tube rack properly labelling the required concentrations of the sample (20,00,000 ppm, 10,00,000 ppm, 5,00,000 ppm, 2,50,000 ppm). Antibiotic and blank control were also kept. 2ml of MHB was added to all the tubes. 2ml of the sample having 40,00,000 ppm concentration was added to the first tube so that the concentration in that tube will be 20,00,000 ppm. Similarly, serial dilution was performed till the required concentration and 2 ml was discarded from the last tube. 0.2 ml of broth culture having a turbidity equivalent to 0. 5 MacFarland unit was added to all the tubes. All the tubes were kept for incubation at 37ºC for 24 hours. After incubation the tubes were visually observed. If the visual observation is not clear due to sample matrix, streak from each doubtful tube to MHA plates and incubate the plates 37ºC for 24 hours. MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube or growth in the plate.

RESULT

Table No.1: Result of Agar well Diffusion test – Zone of Inhibition (in mm) of Kutajadi Churna.

S.no	Organism	Sample				Strepto-mycin (15,000 ppm)	Solvent control-Methanol
		2,00,000 ppm	1,00,000 ppm	50,000 ppm	25,000 ppm		
1.	*E. coli*	No Zone	No Zone	No Zone	No Zone	30 mm	No Zone
2.	*S. typhi*	No Zone	No Zone	No Zone	No Zone	32 mm	No Zone
3.	*S. flexineri*	No Zone	No Zone	No Zone	No Zone	32 mm	No Zone
4.	*S. aureus*	14 mm	11 mm	No Zone	No Zone	32 mm	No Zone

Table No.2: Result of MIC of Kutajadi Churna- Broth Dilution Method.

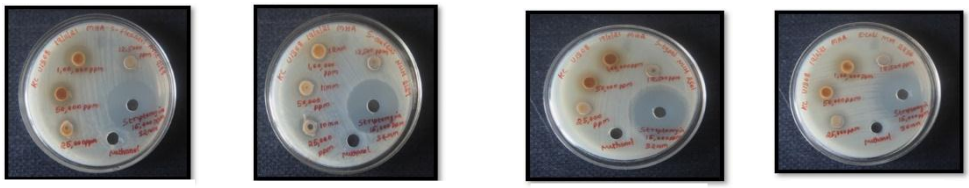
S.no	Organism	Sample				Streptomycin15,000ppm	Blank control
		2,00,000 ppm	1,00,000 ppm	50,000 ppm	25,000 ppm		
1.	*E. coli*	No growth	Growth	Growth	Growth	No growth	Growth
2.	*S. typhi*	No growth	No growth	Growth	Growth	No growth	Growth
3.	*S. flexineri*	No growth	No growth	Growth	Growth	No growth	Growth
4.	*S. aureus*	No growth	No growth	No growth	Growth	No growth	Growth

Table No.3: Result of Agar well Diffusion test – Zone of Inhibition of Kutajadi Suspension.

S.no	Organism	Sample				Streptomycin (15,000 ppm)
		20,00,000 ppm	1,00,000 ppm	50,000 ppm	25,000 ppm	
1.	*E. coli*	No Zone	No Zone	No Zone	No Zone	30 mm
2.	*S. typhi*	No Zone	No Zone	No Zone	No Zone	33 mm
3.	*S. flexineri*	18 mm	No Zone	No Zone	No Zone	32 mm
4.	*S. aureus*	14 mm	No Zone	No Zone	No Zone	35 mm

Table No.4: Result of MIC of Kutajadi Suspension- Broth Dilution Method.

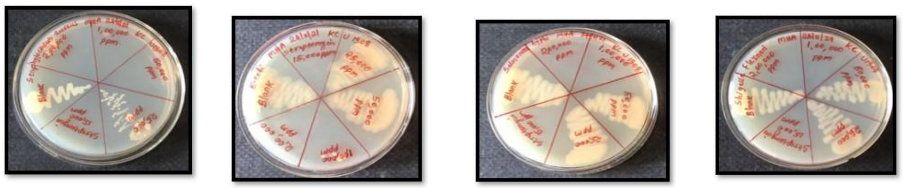
S.no	Organism	Sample				Streptomycin (15,000 ppm)	Blank control
		20,00,000 ppm	10,00,000 ppm	5,00,000 ppm	2,50,000 ppm		
1.	*E. coli*	No growth	Growth	Growth	Growth	No growth	Growth
2.	*S. typhi*	No growth	Growth	Growth	Growth	No growth	Growth
3.	*S. flexineri*	No growth	Growth	Growth	Growth	No growth	Growth
4.	*S. aureus*	No growth	Growth	Growth	Growth	No growth	Growth



(a) *S. aureus* **(b) *S. flexneri*** ***(c) S. typhi*** ***(d) E. coli***

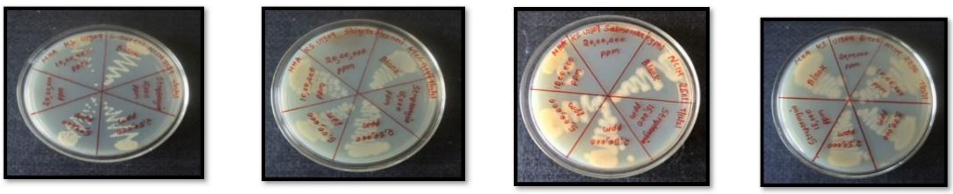
Fig 1: Agar Well Diffusion of Kutajadi Churna.

Fig 2: MIC of Kutajadi Churna.



(a) *S. aureus* **(b) *S. flexneri*** ***(c) S. typhi*** ***(d) E. coli***

Fig 3: Agar Well Diffusion of Kutajadi Suspension.



(a) *S. aureus* **(b) *S. flexneri*** ***(c) S. typhi*** ***(d) E. coli***

Fig 4: MIC of Kutajadi Suspension.

DISCUSSION

Microbiology is a field of biology that studies microorganisms that determines the presence of bacterial growth using the agent of interest and infects bacteria in the in-vitro setting to determine the antimicrobial activity of the test sample. The resistance or sensitivity of the organism to the tested substance is determined by bacterial growth.[8]

Agar well diffusion method **i**s by inoculating the agar plate with microbial inoculum, this method is commonly used to check antimicrobial activity of any test sample. Broth dilution method (minimum inhibitory concentration) does comparing bacterial culture to 0.5 Mc Farland opacity standards, the bacterial inoculum is created. In test tubes, serial dilutions of the medicine in broth are taken, and a standardised suspension of the test bacterium is introduced where the minimum inhibitory

concentration (MIC) is the lowest concentration of antimicrobial agent at which no observable growth occurs.[9]

Preliminary phytochemical screening of kutajadi churna reveals the presence of active phytoconstituents like carbohydrate, reducing sugar, monosaccharides, hexose sugar, steroids, flavonoids, tannins, glycosides like cardiac glycoside, anthraquinone, saponin glycosides. Steroids are responsible for regulating the immune response.[10] Flavonoids possess properties like antimicrobial, anti-inflammatory, antioxidant, antimicrobial activity.[11] Tannin containing drugs are proven for astringent which are beneficial in treating diarrhoea, stomach, and duodenal tumours,[12] also act as anti-inflammatory, antioxidant.[13] Saponin are immunostimulant, kill protozoans,[14] antioxidants help in digesting protein and uptake of vitamins and minerals present in the gut, also as antifungal and antiviral.[15]

The Kutajadi churna and Kutajadi suspension were taken for antimicrobial study by Agar well diffusion method and MIC determination by Macro Broth Dilution Method using gram positive and gram-negative microorganisms like *Staphylococcus aureus, E. coli, Salmonella typhi* and *Shigella. flexineri* that are responsible for infectious diarrhea[16], bloody diarrhea, dysentery.

In the present study, both Kutajadi churna and Kutajadi suspension has shown sensitivity for *E. coli, S. typhi, S. flexineri and S. aureus* under various concentrations. Kutajadi churna shown zone of inhibition of 14 mm in 2,00,000 ppm and 11 mm in 1,00,000 ppm for *S. aureus*. MIC shown no growth for *E. coli* (2 lac ppm), *S. typhi* (2 lac and 1 lac ppm), *S. flexineri* (2 lac and 1 lac ppm), *S. aureus* (2 lac,1 lac and 50,000 ppm) (shown in table no. 1, 2 and Fig 1 and 2). Kutajadi suspension shown zone of inhibition of 18 mm for *S. flexineri* (20,00,000 ppm) and 14 mm for *S. aureus* (20,00,000 ppm). MIC shown no growth for *E. coli, S. typhi, S. flexineri* and *S. aureus* (20 lac ppm) (shown in table no. 3, 4 and Fig 3 and 4).

Kutajadi churna shown inhibition for one organism i.e., *S. aureus* whereas Kutajadi suspension shown inhibition for two organisms i.e., *S. flexineri* and *S. aureus* which may be because of add on effect of Kutajadi suspension when mixed with its anupana honey.

Moreover, all the drugs of Kutajadi churna are proven for its antimicrobial, antidiarrheal, antioxidant, anti-inflammatory activity. Mahato S et.al. studied antibacterial activity of kutaja against *E. coli, Staphylococcus aureus, E. coli, Salmonella typhi* and found that bark extract shown maximum activity against *Staphylococcus* (10.05 mm inhibition zone) followed by *Salmonella* (6.65mm) and *E. coli* (2.7mm).[17] Dey A et.al. stated that water and alcoholic bark extracts of kutaja act against EIEC (enteroinvasive *E. coli*), Shigella flexneri, Shigella boydii and Salmonella enteritidis which are the major causative organism for diarrhea.[18] Shrestha et al. concluded that the antimicrobial activity results shown exhibited antimicrobial activity and susceptibility against *E. coli* and *S. aureus* by the *Cissampelos pareira* extract.[19] Siddaraju M et.al. concluded, the gastroprotective activity of aq. extract of shunti as it protects gastric mucosa from stress induced mucosal lesions and could inhibit the gastric acid secretion by blocking H+, K+-ATPase action, also by inhibiting growth of H. pylori which shows the anti-oxidant effect against oxidative stress-induced gastric damage.[20] S Brijesh et.al. could validate the classical action in diarrheal diseases especially infectious diarrhoea by this study. The antimicrobial activity of kashaya of the bilwa apakwa had limited activity against selected microbes, but it could retard colonization of bacteria to gut epithelium and production and action of few enterotoxins.[21] Kumaraswamy MV et.al studied *W. Fruticosa* for antimicrobial activity and found that different extracts of dried flowers of *W. Fruticosa* have significant antibacterial activity against fourteen gram-positive and negative human pathogens.[22] Aljadi AM et.al. studied honey for antioxidant activity and found that it contains both aqueous and lipophilic antioxidants which enable it to act at different cellular levels as an ideal natural antioxidant.[23]

Therefore, the present study and the supportive research puts lights on the antimicrobial activity of Kutajadi churna its suspension and raw drugs of the formulation. Also, by it's synergistic effects will contribute in treating the indicated disease along with its associated complaints.

CONCLUSION

The phytochemical study shown the presence of metabolites like tannin, flavanoids, saponins, steroids which exhibits the properties like antimicrobial, antidiarrheal, anti-inflammatory, antioxidant. Kutajadi churna and Kutajadi suspension possess antimicrobial activity against *E. coli, S. typhi, S. flexineri,* and *S. aureus* at various concentrations. Thus, proving the antimicrobial action of Kutajadi churna and its modified form Kutajadi suspension.

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