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RESEARCH ARTICLE

# Assessment of antimicrobial and invitro antacid activities of kolakhar: an indigenous herbal soda of Assam, India

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## ABSTRACT

Kolakhar (KK) is a traditionally used soda of Assam. KK is widely used as a detergent or soaps from ancient time to wash cloths and hair. In the rural part of Assam, KK is familiar to treat stomach disorder, respiratory tract disorder and as an antibacterial agents. It is also used as pesticides in different agricultural fields. The present study investigates the antimicrobial and antacid effect of the kolakhar by using disc diffusion method and a modified artificial stomach model. The observation of antimicrobial activity, kolakhar on human pathogenic species using disc diffusion method showed that the kolakhar have more impact on S. aureus (21.33 mm). The neutralisation effect, duration of neutralisation effect and capacity were found to be higher for kolakhar than sodium bicarbonate.

## INTRODUCTION

*Kolakhar* is a ethinic herbal soda which is mainly prepared from the banana plant (Musa balbisiana colla.). *Kolakhar* (KK) is a traditional used soda of Assam. KK is widely used as a detergent or soaps from ancient time to wash cloths and hair. Traditionally, it is used as a food additives, especially as a boiling agents. In the rural part of Assam, KK is familiar to treat stomach disorder, respiratory tract disorder and as an antibacterial agents. It is also used as a pesticides in different aggricultural fields .Peoples from the different rural areas of Assam, believed that this product also having the antacid activity.

Different parts of banana tree are used to prepare the natural soda commonly known as *"kolakhar"* in Assam, India. Rhizomes, stem peels are commonly used for this preparation. In general, Kolakhar prepared from Athiya kol (*Musa balbisiana*) was observed to be redish in colour and more dense compared to the *Kolakhar* obtained from different species of Banana plants . People believed that *khar* obtaining from athiya kol is more effective than others[1].

A number of studies going on worldwide about the antimicrobial properties of plants. After investigation many have been used as therapeutic alternatives because of their antimicrobial properties. Plants have many antimicrobial properties as secondary metabolites such as alkaloids, phenolic compounds, etc. In developing countries the practice of complementary and alternative medicine is increase now days on scientific basis for the efficacy of many plants used in folk medicine to treat infections [2-5].

There is a balance between defensive and aggressive factors in normal stomach mucosa. The ulcer etiologies are not yet confirmed but it is generally accepted that peptic ulcers develop when aggressive factors (endogenous, exogenous and/or infectious agents) overcome mucosal defense mechanisms. Some of the main aggressive factors are gastric acid, abnormal motility, pepsin, bile salts, free radicals, use of alcohol and NSAID as well as infection with microorganisms. On the other hand defensive factors such as mucus secretion, bicarbonate production, gastroprotective prostaglandin synthesis, endogenous nitric oxide and normal tissue microcirculation protect against ulcer formation[6-7]. Recently some approaches to control peptic ulceration include potentiation of the mucosal defense along with reduction of acid secretion and its neutralization, enhancement of antioxidant levels in the stomach, stimulation of gastric mucin synthesis and inhibition of *H. pylori* growth[7-10]. The currently used drugs produce many adverse effects. Due to the alkalinity nature of *kolakhar*, traditionally the peoples of Assam are using this product to neutralize the acidity.

Keeping in view the use of *kolakhar* as an effective home remedy for acidity, the present study was undertaken to evaluate the the antimicrobial activity and antacid effect of *kolakhar* .

## MATERIALS AND METHODS

### Plant material

The whole plant of *Musa balbisiana* was collected locally from village Ischadagharia,Kamrup, Assam, India. The plant materials were identified and authenticated taxonomically by an expert taxonomist of Guwahati University, Assam, India (Authentication No- 17891).

**Preparation of *kolakhar*-**

Clean the plant parts with normal water to remove the unwanted materials such as soil. Then slice the different parts in to smaller size and dried in proper sunlight. Then the plant materials was subjected for ignition in open air to convert it to ash. A mixture of 25 gm dry ash of whole plant of *Musa balbisiana* and 500ml of distilled water taken in a one litre conical flask was stirred magnetically for one hour. After filtering, the residue washed with distilled water. The filtrate (light yellow colour) is known as *kolakhar*. Then the *kolakhar* is concentrated by evaporation. Dried extracts were kept in refrigerator and used for further study.

**Chemicals and reagents**

All the chemicals and reagents were purchased from Sigma Chemical Co., St Louis, MO, USA. ,Merck Ltd., Mumbai, India. S.D. Fine Chemicals Ltd., Mumbai, India. All other chemicals were obtained from local sources and were of analytical grade.

**Instruments**

The instruments used in this experiment were a standard pH meter (LABINDIA, SAB 5000), a magnetic stirrer with hot plate temperature controller (1MLH, REMI), an adjustable electrode stand and a peristaltic tubing pump (ELECTROLAB PP 201 V).

**Dose consideration-**

Doses of *kolakhar* were taken by considering average animal weight 200gm and dose is 250 mg/kg (kolakhar-I) & 500mg/kg (kolakhar-II).

**Assay of antimicrobial activity using Disc diffusion method [2-5]**

The 20 ml of sterilized Muller Hinton Agar was poured into sterile petriplates, after solidification, 100 µl of fresh culture of human pathogens were swabbed on the respective plates. The discs were kept over the agar plates using sterile forceps at various concentrations. The plates were incubated for 24 hours at 37C. After incubation the diameter of inhibitory zones formed around each discs were measured (mm) recorded.

**Preparation of artificial gastric acid[6-8]**

Two grams of NaCl and 3.2 mg of pepsin were dissolved in 500 mL distilled water. Hydrochloric acid (7.0 mL) and adequate water were added to make a 1000 mL solution. The pH of the solution was adjusted to 1.20.

**$P^H$ determination of *kolakhar* [6-8]**

The pH of *kolakhar* (kolakhar-I & kolakhar-II) was determined at temperatures ranging from $25^0$C to $37^0$C. The pH values of the active control solution sodium bicarbonate (SB) and water was also determined for comparison.

**Determination of the neutralizing effects on artificial gastric acid [7-9]**

The freshly prepared test solutions *kolakhar*, water (90 mL) and the active control SB (90 mL) were added separately to the artificial gastric juice (100 mL) at pH 1.2. The pH values were determined to examine the neutralizing effects on artificial gastric juice. (Six experiments were performed for each solution)

**Determination of the duration of consistent neutralization on artificial gastric acid using the modified model of Vatier's artificial stomach[6-10]**

The apparatus of the modified model of Vatier's artificial stomach was made up of three elements: a pH recording system (R), a stomach (S) and a peristaltic pump (P). The stomach was made up of three portions, S1, S2 and S3. S1 was a reservoir (container), S2 modeled the secretory flux (F-IN), and S3 modeled the gastric emptying flux (F-OUT). Each freshly prepared test sample (90 mL) was added to 100 mL of artificial gastric juice at pH 1.2 in the container of the artificial stomach at 370C and continuously stirred (30 rpm) with a 2.5-cm magnetic stirring apparatus. Artificial gastric juice at pH 1.2 was pumped at 3 mL/min into the container of the artificial stomach, and pumped out at 3 mL/min at the same time. A pH meter was connected to continuously monitor the changes of pH in the container of the artificial stomach. The duration of the neutralization effect was determined when the pH value returned to its initial value (pH 1.2). Six experiments were performed for each freshly prepared test solution, water and standard (SB).

**Determination of the neutralization capacity *in vitro* using the titration method of Fordtran's model** [6-10]

Each freshly prepared test sample (90 mL) was placed in a 250 mL beaker and warmed to 370C. A magnetic stirrer was continuously run at 30 rpm to imitate the stomach movements. The test samples were titrated with artificial gastric juice to the end point of pH 3. The consumed volume (V) of the artificial gastric juice was measured. The total consumed H+ (mmol) was measured as 0.063096 (mmol/mL) × V (mL). Six experiments were performed for each freshly prepared test solution, water and standard (SB).

Table1. Antimicrobial Activity of *Kolakhar*

| Pathogen | Drugs | Zone of inhibition (mm) |
|---|---|---|
| *S. aureus* | Distilled water | -- |
| | Standard (ciprofloxacine);10 µg/disc | 25.33±0.23 |
| | Kolakhar; 250µg/disc | 15.67±0.14 |
| | Kolakhar; 500µg/disc | 21.33±0.32 |
| *P. aeruginosa* | Distilled water | -- |
| | Standard (ciprofloxacine);10 µg/disc | 24.00±0.15 |
| | Kolakhar; 250µg/disc | 16.67±0.26 |
| | Kolakhar; 500µg/disc | 19.33±0.21 |
| *C. albicans* | Distilled water | -- |
| | Standard (fluconazole);25µg/disc | 26.33±0.13 |
| | Kolakhar; 250µg/disc | 14.00±0.16 |
| | Kolakhar; 500µg/disc | 18.67±0.18 |

Table 2. $P^H$ values with 90 ml water, standard and *kolakhar* added to 100 mL of artificial gastric juice

| SI NO. | DRUGS | $P^H$ |
|---|---|---|
| 1 | Water | 1.54 ± 0.02 |
| 2 | Standard | 2.24 ± 0.16** |
| 3 | Kolakhar-I | 1.94 ± 0.06** |
| 4 | Kolakhar-II | 1.86 ± 0.02* |

Data are presented as mean ± SEM ($n$ = 6) *$P$ < 0.05, **$P$ < 0.01 when compared with water

**Table 3.** Duration of antacid effect for consistent neutralization of gastric acid

| SI NO. | DRUG | Time(min) |
|---|---|---|
| 1 | Water | 106 ± 4.03 |
| 2 | Standard | 188 ± 8.67* |
| 3 | Kolakhar-I | 174 ± 2.31* |
| 4 | Kolakhar-II | 162 ± 7.63* |

Data are presented as mean ± SEM ($n$ = 6) *$P$ < 0.01 when compared with water

**Table 4**. Consumed volume of artificial gastric juice and H+ (mmol) in the titration of 90 mL water, standard and *Kolakhar* with artificial gastric juice ( pH 1.2) to the end point of pH 3

| SI NO. | DRUG | Consumed volume of artificial gastric juice (mL) | **mmol of $H^+$** |
|---|---|---|---|
| 1 | Water | 2.42± 0.13 | 0.14± 0.006 |
| 2 | Standard | 70.14± 0.118* | 3.75± 0.08* |
| 3 | Kolakhar-I | 20.17± 0.75* | 1.30± 0.05* |
| 4 | Kolakhar-II | 19.33± 0.92* | 1.15± 0.06* |

Data are presented as mean ± SEM ($n$ = 6) *$P$ < 0.01 when compard with water

## Results-

### Antimicrobial activity-

*Kolakhar* showed a good antimicrobial activity against *S. aureus*, *P. aeruginosa* and *C. albicans*. These three different pathogens have tested with commercially available antibiotics and results were indicated in Table 1. All the test concentrations used against the pathogenic organisms have showed varied degree of antimicrobial activity against the pathogens.

### At different temperature (25 to 37), ph values of test samples-

*Kolakhar* prepared from M. balbisiana was found to have very high pH (pH 12.0) at temperatures from $25^0$ c to $37^0$ c. at temperatures from 250C to 370C , the pH values of water and SB solutions ranged from 6.92 to 7.23 and 9.23 to 9.27, respectively. From this value we can tell that temperature did not affect pH significantly.

### Neutralizing capacities of *kolakhar* on artificial gastric acids-

After adding of 90 mL of the test solution with 100 mL of the artificial gastric juice (pH 1.2), the pH valuesv of kolakhar–I and kolakhar–II solutions were found to be 1.94 ± 0.06 and 1.86 ± 0.02, respectively. The pH values of water and SB solutions were compared with ph values of *kolakhar* at differet concentration. This result shows that the neutralizing effect of *kolakhar* was significantly better than that of water.

### Duration of consistent neutralization effect on artificial gastric acids-

Neutralizing effects of *kolakhar* solutions were last for 174 ± 2.31 min and 162 ± 7.63 min, respectively. Duration of consistent neutralization effect of water and SB solutions were found 106 and 188 min, respectively. The duration of antacid action of SB was the longest, followed by the *kolakhar* which were significantly higher than that for water.

### *In Vitro* Physical neutralization capacity

The consumed volumes of artificial gastric juices to titrate to pH 3.0 for water,

kolakhar-I, kolakhar-II and SB solutions were 2.42, 20.17, 19.33 and 70.14, respectively.The consumed $H^+$ were 0.14, 1.30, 1.15 and 3.75 mmol, respectively. The active control SB and both tests exhibited significant antacid potency.

## Discussion-

Infectious diseases are the major cause of morbidity and mortality worldwide. The number of multidrug resistant microbial strains and the appearance of strains which reduced susceptibility to antibiotics are continuously increasing. Such increase has been attributed to indiscriminate use of broad spectrum antibiotics, immunosuppressive agents, intravenous catheters organ transplantation and ongoing epidermis of human immunodeficiency virus (HIV) infections. This situation provided the impetus to the search for new antimicrobial substances from various source like medicinal plants.
Stomach is an organ which undergoes propulsion, mixing of food, digestion and absorption of food along with the secretary functions. The parietal cells of the stomach secrete about 2500mL of the gastric juice daily. The acid in this gastric juice kills many bacteria and provide a low pH for pepsin to start protein digestion. Mucosal erosions or ulcerations take place when aggressive factors overwhelm the defensive factors of the gastrointestinal mucosa. This leads to the arrival of gastritis, peptic ulcer and gastro oesophageal reflux disease. The main aggressive factors well established for several decades are acid and pepsin. Hence peptic ulcer diseases are mostly treated with antacids, H2 receptor antagonists and proton pump inhibitors.

In this present study, preliminary screening for antimicrobial activity showed that the *kolakhar* exhibited maximum inhibitory zone (30 mm) against *Staphylococcus* and *kolakhar* showing the good potential as an antacid. In our laboratory *in vitro* study also found the considerable antacid activity of *kolakhar*. With more investigation and proper scientific formulation of *kolakhar* may be give a good herbal antacid and anti microbial agents.

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## References-

1. Deka DC, Talukdar NN. 2007. Chemical and spectroscopic investigation of kolakhar and its commercial importance. Indian J Trad Knowledge. 6(1),72-78.
2. IE Oboh, JO Akerele, O Obasuyi, 2007. Antimicrobial activity of the ethanol extract of the aerial parts of *sida acuta* burm.f. (malvaceae). Tropical Journal of Pharmaceutical Research. 6 (4), 809-813.
3. Rajesh Kowti, Harsha. R, Mohammed Gulzar Ahmed, Hareesh AR, Thammanna Gowda SS, Dinesha R, Satish Kumar BP, Irfan Ali M, 2010 . Antimicrobial activity of ethanol extract of leaf and flower of *Spathodea campanulata P*. Beauv. RJPBCS .1 (3 ) ,691-698.
4. Ibtisam Mohammed Ababutain, 2011. Antimicrobial Activity of Ethanolic Extracts From Some Medicinal Plant. Australian Journal

of Basic and Applied Sciences. 5(11), 678-683.

5. M. Mashiar Rahman, M. Mominul Islam Sheikh,Shamima Akhtar Sharmin1, M. Soriful Islam1,M. Atikur Rahman, M. Mizanur Rahman, M. F. Alam,2009. Antibacterial Activity of Leaf Juice and Extracts of *Moringa oleifera* Lam. against Some Human Pathogenic Bacteria.CMU.J.Nat.Sci.8(2),219-227.

6. Vandana Panda, Prashant Khambat, 2013. Evaluation Of Antacid Activity Of *Garcinia Indica* Fruit Rind By A Modified Artificial Stomach Model. Bulletin Of Environment, Pharmacology And Life Sciences.2(7), 38- 42.

7. Sandhya S, Venkata Ramana K, Vinod K R, Chaitanya Rsnakk,2012. Assessment of in vitro Antacid Activity of Different Root Extracts of Tephrosia purpurea (L) Pers by Modified Artificial Stomach Model. Asian Pacific Journal of Tropical Biomedicine.2012, S1487-S1492.

8. Sandhya S, Venkata Ramana K, Vinod K.R, 2015. A Comparative Evaluation Of *In Vitro* Antacid Activity Of Two *Tephrosia* Species Using Modified Artificial Stomach Model. *Hygeia.J.D.Med.*7 (2); 9-17.

9. Sandhya S. Gouthami gade. Vinod kombath ravindranvidya SE,2012. Invitro and invivo evaluation of the inhibitory effect of limnophila indica (linn.) druce on shigellosis.journal of Chinese integrative medicine.10(5),538-545.

10. Tsung-Hsiu Wu, I-Chin Chen, Lih-Chi Chen,2010. Antacid effects of Chinese herbal prescriptions assessed by a modified artificial stomach model. *World J Gastroenterol.* 16(35), 4455-4459.