

# Antimicrobial efficacy of potential plants used in the indigenous preparation of traditional rice beverage “Handia”

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## Abstract

The context and purpose of the study: To explore the available plants used for starter preparation of Handia, their ethnomedicinal uses and to screen phytochemical constituents for antibacterial activity against enteric pathogens.

The main findings: Semi-structured interview was carried out with 24 informants (mean age 46, male) from 24 locations. Qualitative phytochemical analysis, agar cup assay, micro-dilution method for MIC and MBC were followed to study antibacterial properties against eight enteric pathogens. Ethanol extracts of the plants contain abundant alkaloids, flavonoids, carbohydrate, protein and amino acids, saponins, tannin and phenolic compounds. With the exception of *Asparagus racemosus* (root), *Cissampelos pareira* (leaf), *Dioscorea* sp. (tuber), *Rauwolfia serpentina* (leaf) extracts, all other plants exhibited antibacterial activity by agar cup method. The zone of inhibition was found maximum against *Staphylococcus aureus* followed by *Shigella sonnei* and *S. flexneri*. The MIC result ranged from 125 to 1000 µg/ml (w/v) with the lowest against *S. aureus* (125, 156, 250 µg/ml) followed by *S. sonnei* (156, 250, 312, 500, 625 µg/ml). MBC test validate that in between 1000-2500 µg/ml (w/v) concentrations, most of test bacteria were killed due to broad spectrum activity.

Brief summary and potential implications: The study establishes that the traditional knowledge of Handia preparation using different plant parts will be a useful lead for phytochemist and pharmacologists for further study.

**Keywords:** Bakhar or ranu tablets, Rice beer, Fermented food, MIC, Enteric pathogens, Traditional knowledge.

## Introduction

The last few decades have witnessed a renewed interest in the field of ethnopharmacology.[1] Modern scientific research in the field of ethnobotany and ethnopharmacology has validated the traditional use of many plants for various ailments by different cultures.[2] Worldwide the different cultures use the traditional remedies for curing of several diseases. Consultation of traditional healers and use of the herbal medicine recommended by the traditional people, play an integral role in the rural area of India. The market in traditional medicines is expanding and traditional practices are increasingly becoming familiar. A key objective of the scientific study of ethnopharmacology practices is the development and promotion of effective medicines based on inexpensive locally available plants. At the same time the value of conventional medicines in skirmishing infectious diseases is unquestionable for common ailment such as diarrhea, skin infection, intestinal worms, wounds and gynecological disorders.

In India, from time immemorial, both fermented and distilled beverages have been prepared by fermenting different varieties of rice. These beverages are primarily prepared and used by different tribal communities all over the state. Santals constitute the largest

tribal group of the District, Mayurbhanj, Odisha and are scattered throughout the state. The social, cultural and religious life of aboriginal people is influenced by the nature and natural resources available in and around their habitat, provides food, fodder, medicine, shelter and various other material and cultural needs. The fermented food, locally known as Handia, is an inseparable food item in the life of tribals of Mayurbhanj and most other districts of the state. The process of starter (locally known as Bakhara/Ranu) preparation of each tribe is almost similar with slight differences. The main ingredients of starter preparation are rice along with some of the locally available plant parts through some indigenous method. So, a survey of the use of medicinal plants in preparation of Bakhara/Ranu by the tribes in the District Mayurbhanj was carried out. The tribals do not know the authentic role of these plants in the fermentation. According their knowledge, either yeast is formed from these plants or these plants are responsible for the yeast's action in fermentation. The present paper deals with the description and ethnomedicinal uses of plants for the starter preparation by the tribes of Odisha. For scientific validation all these plants were subjected for screened of antimicrobial activity and phytochemical screening.

## Materials and methods

### Survey and documentation of plants

Survey was conducted in different villages of Mayurbhanj district of Odisha and gathered information through questionnaires and personal interaction with native tribal people regarding the use of plants and plant parts in the preparation of starter culture and its detail method of preparation is recorded. As most of the tribals obtain the principal constituents in form of the powder plant parts from the market, so a survey was conducted by showing the plant parts along with their dried form. Accuracy of the information was ensured through cross verification. The plant specimens used in Bakhar preparation were collected, identified and deposited as voucher specimen in Department of Botany, North Orissa University. Medicinal uses of the same plants were obtained by interviewing (once only) traditional healers of 24 villages of the district (Figure-1). All of them were males with an average age of 46 years. Prior informed consent was not taken from the informants as most of them were professionals and reputed in their respective areas and prescribed plant preparations for different ailments.

### Collection and processing of plants

Bark, flower, leaves, roots, rhizome and young shoot of plants have separately been collected during field trips to different places of Mayurbhanj. The roots are dug out from the soil and the adhering soils were removed by shaking and washing. The leaves were plucked from the trees, washed properly and infected leaves were discarded. After collection, the healthy leaves were dried at room temperature to maintain their green color and volatile oils, if present. The material is completely shed dried so long it does not allow for the growth of any type of fungi, molds, bacteria and other microorganisms. The dried bark, flower, leaves, roots and rhizome are powdered separately by using mortar and pestle.

### Extraction of plants

Hundred grams of each powdered samples were dissolved in 200ml of ethanol separately in wide mouth bottle. The suspension was then filtered (Whatman No. 40) separately and utilized for studying antimicrobial properties and phytochemicals. Ethanol extract was dried in rotary evaporator (Sonax, India) at 40 C and store in refrigerator for further study.

### Phytochemical Analysis

Qualitative phytochemical analysis was carried out using methods described by Trease and Evans.[3] Each extract was screened for presence of alkaloids (using Mayer's, Wagner's, Hager's and Dragend dorff's reagents); flavonoids (NaCl and HCl); carbohydrates (using Molisch's reagent); glycosides (using Keller Killiani and Borntrager's reagents); protein and amino acids (using Biuret, Xanthoproteic, Ninhydrin and Millon's reagent); tannin and phenolic compounds (FeCl<sub>3</sub> and Gelatin); triterpenoids (thionyl

chloride solution); steroid and sterols (using Liebermann Burchard and Salkowski's reagents), fat and fixed oils with alcoholic KOH reagents.

### Antimicrobial activity

Enteric pathogens viz. enteropathogenic and enterotoxigenic *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella flexneri*, *S. sonnei*, *Staphylococcus aureus* and *Vibrio cholerae* were used in the present study as discussed earlier by Panda et al.[4] The antibiogram was carried out by adopting disc diffusion method using several antibiotics. The result of the antibiogram was published in our earlier report.[4] The agar cup method and MIC were used to study the antibacterial activity. Broth microdilution technique adopted using 96-well microtiter plate and tetrazolium salt, 2,3,5-triphenyltetrazolium chloride (TTC), was carried out to determine the MIC following the method as described by Eloff et al.[5] Selected extracts were serially diluted in the 96-well plate with an overnight culture of microorganisms (0.5 McFarland) grown at 37 C to obtain the final concentration of extracts ranging from 78 to 2500 µg/ml. The microplate was sealed and incubated at 37 C and observed for the growth of the microorganism. 10µl of the broth from each well of 96 microtiter plate (MIC) and control wells were taken aseptically and plated on one day old MH agar plate as a point inoculum and allowed to dry for 10 min. under the laminar air hood. These plates were then sealed and incubated at 37 C for 24 hours and observed for growth of the bacteria. Absence of growth of the bacteria showed the MBC result of the respective bacteria.

## Results

### Ethnomedicinal uses, Preparation of *Bakhar* or *Ranu* tablets

*Ranu* or *bakhar* tablets act as starter for fermentation. *Ranu* tablets are mixtures of various plant parts (50%) and powdered un-boiled rice (50%). The plant species and parts thereof used for the purpose along with local names, family, parts used and their ethnomedicinal uses are listed in Table-1 and Figure-2. Some species viz. *Asparagus racemosus* (Willd.), *Cissampelos pareira* L. var. *hirsuta* (DC) Forman, *Clerodendrum serratum* (L.) Moon, *Coccinia grandis* (L.) Voigt, *Holarrhena antidysenterica* Wall ex. A. DC., *Woodfordia fruticosa* (L.) Kurz. and Benth. are commonly used by the tribal of all localities while plants such as *Madhuca longifolia* (Koenig), *Smilax macrophylla* (Roxb.), *Rauwolfia serpentina* (L.), *Elephantopus scaber* L., *Gardenia gummifera* L.f. and *Dioscorea* sp. are rarely use. Depending on the season and availability in a particular locality, plant parts of one or more species are used. The accurate ratio of different plants used for *ranu* preparation could not be ascertained as the informants were reluctant to disclose the same. However, *C. pareira* forms the major part in most of the preparations (70%) followed by other plants in combination (1-30%). *R. serpentina* and *G. gummifera* are used in very small proportion. According to one informant

(Sama Singh, Male, Age-62) the ratio of the plant (root) is 6:2:1:1 (*C. pareira*, *W. fruticosa*: *A. racemosus*, *H. antidysenterica*). Preferred parts and plants varied at different places. Dried root, stem and other parts used for the purpose, both as such and powdered, are abundantly and openly sold in the local markets (Figure-4. a & b). Powdered plant ingredients are mixed with equal amount of rice (*Oryza sativa* L.) powder. A suitable amount of water is added to make dough. *Ranu* is prepared in the form of rounded tablets and spread over straw beds in layer over layer with a final thin layer of straw cover. After 3 days, the *ranu* tablets are picked up from straw beds and dried under sun for about 2 days and stored for use in fermentation of rice beverages (Figure- 3. e). These tablets are not only used for fermenting rice beverage but also used for treatment of various ailments. A paste of *ranu* tablets with saliva is applied on mumps by the tribes before sleeping to get relief. Santal tribals also disinfect silk worm (tasar) eggs during indigenous rearing. From the study conducted in the various villages, it is evident that all tribes use some plants for starter preparation; however the type of plant varies from tribe to tribe and village to village. The tribal peoples of Sukruli and Karanja block use the same plant viz. *C. pareira*, *C. serratum*, *C. grandis*, *Dioscorea* sp. for starter preparation. Similarly tribes of Bijatola and Rairangpur use the same plant in addition to *A. racemosus* instead of *Dioscorea* sp. However, the tribes of Samakhunta use different plants *A. racemosus*, *C. pareira*, *H. antidysenterica*, *M. longifolia*, *S. macrophylla* and *W. fruticosa* for the same purpose. This is an age old practice in these communities which is followed generation after generation.

### Screening of phytochemicals and antimicrobial activity of plants parts used in starter

The qualitative phytochemical analysis of ethanol extracts indicated the presence of alkaloid, flavonoid, carbohydrate, protein and amino acid, tannin and phenolic compounds and saponin in most of the plants (Table-2). However, glycoside, steroid & sterols, gum & mucilages, oil & fats and triterpenoids were found in less number of test plants. Alkaloid, carbohydrates, and saponin are universally present in all test plant extracts. Preliminary screening of antimicrobial activity was evaluated by using agar cup method against eight human pathogenic bacteria are given in Table-3. The zone of inhibition was found maximum against *S. aureus* followed by *S. sonnei* and *S. flexneri*. Organism such as *S. typhimurium* and *V. cholerae* show moderate zone of inhibition while enteropathogenic and enterotoxigenic *E. coli* and *P. aeruginosa* show least zone of inhibition. No test organisms were inhibited by *A. racemosus* (root), *C. pareira* (leaf), *Dioscorea* (tuber), *R. serpentina* (leaf) extract. So these plant extracts are not subjected to further study on MIC and MBC. The MIC results among all test bacteria are summarized (Table-4) and showed that extracts were able to prevent the growth of most of the test strains with selective activities. The growth inhibition of the test bacteria ranged from 125 µg/ml (w/v) to 1000 µg/ml (w/v) with the lowest MIC value against *S. aureus* (125, 156, 250 µg/ml) followed by *S. sonnei* (156, 250,

312, 500, 625 µg/ml). MBC test showed that in between 1000-2500 µg/ml (w/v) concentrations, most of test bacteria were killed.

### Discussion

Plant ingredients are used in preparation of starter culture (*ranu* or *bakhar*) universally that are essential for fermentation of rice to prepare beverages. After surveying 12 districts of Odisha including Mayurbhanj, Dhal et al.[6] recorded the use of bark and root of six plant species in the preparation of *bakhar* by the tribals. Singh[7] has reported the use of several plant species viz. mature leaves of *Allophylus cobbe* (L.) Blume, *Antidesma roxburghii* Wall. ex Tul. and tender leaves of *Artocarpus heterophyllus* (Lam.), in equal proportions together with little chilli, are used in the preparation of *Choarak*, a local wine of Tripura state, India. Tribal inhabitants of tea gardens in Terai region of West Bengal use 12 plants, specific parts for particular purpose, for preparation of rice beer *jhara* or *haria*. [8] According to Ghosh and Das[8] Sweetness of the liquor is developed by the use of tuberous roots of *Coccinia grandis* L. (Voigt), whole plant including fleshy roots of *Veronia cinerea* L. (Lessing) and leafy twigs of *Scoparia dulcis* L.. Likewise, young and soft leaves of *Clerodendrum viscosum* Ventinat, bark of *Oroxylum indicum* (L.) Benth. Ex. Kurz, root bark of *Rauwolfia serpentina* (L.) Benth. Ex. Kurz, and bark of *Wattakaka volubilis* (L.f.) Staph. produce a bitter taste. Leafy branches of *Plumbago zeylanica* (L.) act as a process enhancer. Roots of *Stephania japonica* (Thumb.) Miers and *Stephania glabra* (Roxb.) Miers, are used for long storing. Roots of *Mussaenda roxburghii* (Hook.f.) and leaves of *Artocarpus heterophyllus* (Lam.), impart sweetness and yellowish tint to the liquor. The tribals of Central India use a number of roots, bark, rhizomes, leaves and seeds of some 21 plants for making *ranu*. [9] The tribals get the phytotherapeutic value of these plants through the drink. The use of specific plants and parts thereof is a tradition and passed through generations. However, due to the loss of biodiversity and fragmentation of habitat, all the plants are not available in the vicinity of a particular village.

Wani et al.[10] screened presence of phytochemicals in *A. racemosus* root from Himalaya region, India. According their findings, both alcoholic and aqueous extracts confirm presence of carbohydrate, glycoside, mucilage and saponin. In a study conducted by Mandal et al.[11] have shown antibacterial property of methanol extract of *A. racemosus* root against *Escherichia coli*, *Shigella dysenteriae*, *S. sonnei*, *S. flexneri*, *Salmonella typhimurium*, *Pseudomonas putida* and *Staphylococcus aureus*. However, in the present investigation antibacterial activity was not recorded against any test pathogens with the ethanolic extracts of *A. racemosus*.

Jhuma et al.[12] studied phytoconstituents from methanolic extracts of *Cissampelos pareira* flower and result revealed presence of alkaloid, carbohydrate, flavonoid, protein, tannin and terpenoids. In the present study similar results were obtained for presence of phytochemicals in the ethanol extracts except triterpenoids. On the other hand, leaf extract don't show antibacterial activity against any strain while root extract show good inhibitory property against *S. aureus* and *S. sonnei*. This may be





due to presence of certain additional phytochemicals in root compare to leaf.

Prasad et al.[13] reported presence of alkaloid, anthroquinoles, glycoside, phenol, saponin, tannin and terpenoids in *Clerodendrum serratum*. The same authors also evaluate the antibacterial activity of various extracts and result found that isoamyl alcohol had better antibacterial property against *Bacillus subtilis*, *S. aureus*, *S. typhi* and *Proteus* species. Similar type of results was also recorded by Vidya et al.[14] during their study on the same plant. Khatun et al.[15] studied the antibacterial activity of various extracts of *Coccinia grandis* and result found that methanol extracts showed antibacterial activity against *S. aureus*, *S. dysenteriae*, *E. coli* and *S. typhi*. These authors also investigated presence of phytochemicals such as flavonoids, phenols, saponin, tannin and terpenoids. Similar study conducted by Umamaheswari and Chattarjee[16] shown presence of phytoconstituents viz. alkaloid, glycoside, flavonoid, phenols, saponin and tannin. Presence of triterpenoids reported by Syed et al. [17] whereas proteins and aminoacid by Umamaheswari and Chattarjee,[17] while presence of both these phytochemicals in the ethanol extract right through the present study. Povendran et al. [18] reported antibacterial activity of leaf extracts of *C. grandis* against *Helicobacter pylori*. Nevertheless, in the present study the rhizome extract exhibited very good antibacterial activity against *S. aureus*, *S. flexneri* and *S. typhimurium*.

Poli et al.[19] evaluated pharmacological properties of *Elephantus scaber* and observed the presence of alkaloid, anthocyanin, chalcones, flavonoid, lactone and triterpenoids. In another study conducted by Kamalkannan et al.[20] showed presence of alkaloid, carbohydrate, proteins and saponin while absence of volatile oil, gum, mucilage and steroid. The same authors studied the antibacterial property and conclude that the methanol extract of *Elephantus scaber* has potential antimicrobial activity against *Streptococcus pyogenes* while no activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. typhi*. In contrary to this work the study conducted by Kumar et al.[21] and Avani and Neeta[22] reported promising antibacterial activity against pathogens such as *B. subtilis*, *E. coli*, *P. aeruginosa* and *S. aureus*. At this juncture, antibacterial activity was confirmed against *S. aureus*, *S. typhimurium* and *S. flexneri* whereas absence of activity against *E. coli*, *P. aeruginosa*, *S. sonnei* and *V. cholerae*. Tambekar and Khante[23] evaluated antibacterial activity of *Gardenia gummifera* and result originated that this plant exhibit activity against *Enterococcus aerogenes*, *Klebsiella pneumoniae*, *S. aureus* whereas no activity was evidenced against *E. coli*, *P. aeruginosa*, *S. typhi*, *Proteus vulgaris* and *S. typhimurium*. These authors also reported presence of phytochemicals viz. alkaloid, flavonoid, glycoside, steroid, tannin and phenolic compounds whilst absence of carbohydrate, protein and aminoacid. On the other hand, in the present study antibacterial activity was not observed by ethanol extract of *G. gummifera* except *S. aureus* (zone of inhibition 12 mm).

Ballal et al.[24] studied antibacterial activity of *Holarrhena antidysenterica* against enteric pathogens and outcome confirmed

antibacterial activity against EPEC, EIEC, *P. aeruginosa*, *Shigella boydii*, *S. flexneri*, *V. cholerae*, *S. aureus* and *S. typhimurium*. Study conducted by Preethi et al.[25] validated antibacterial activity of *H. antidysenterica* against *B. subtilis*, *E. coli*, *S. aureus* and *S. typhimurium*. Chakraborty and Brantner[26] reported antibacterial activity of steroid alkaloid from stem bark of *Holarrhena pubescens* against *B. subtilis*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *Streptococcus faecalis* and *S. typhimurium*. The present findings correlate with the study conducted by all these authors, relating to the antibacterial activity. Chakma[27] studied antimicrobial activity of *Madhuca longifolia* fruit and conclude that the plant act as a potential agent against *B. subtilis*, *E. coli*, *P. aeruginosa* and *S. aureus*. Later, Gopalkrishnan and Shimpi[28] carried out pharmacological studied on stem bark of *Madhuca longifolia* and result evaluated the presence of starch, protein, terpenoid, glycoside, saponin, tannin while absence of alkaloid, flavonoid and steroid. In the present investigation, antibacterial activity was recorded against *S. aureus* while *E. coli* and *P. aeruginosa* was not inhibited by *M. longifolia* extract. Nevertheless, the extract revealed presence of phytochemicals such as carbohydrate, protein, terpenoid, glycoside, saponin and tannin, and these finding are correlate with the study conducted by Gopalkrishnan and Shimpi.[28]

Harisaranraj et al.[29] examine phytochemicals such as alkaloid, flavonoid, phenolic compounds and tannin in the root extracts of *Rauwolfia serpentina*. Deshmukh et al.[30] studied on antimicrobial activity of indole alkaloids from *R. serpentina* and conclude that root extracts illustrate better antimicrobial activity in compare to leaf extracts against *B. subtilis*, *E. coli*, *S. aureus* and *S. typhimurium*. The same authors at preliminary level investigated presence of phytochemicals viz. alkaloid, tannin, saponin, flavonoid and starch. Deshwal and Vig[31] studied antibacterial activity of *R. serpentina* and proved that ethanol extract showed higher zone of inhibition compare to norfloxacin against *S. aureus*. In the present experiment, ethanol extract of root exhibited antibacterial activity against *S. aureus*, *S. sonnei*, *S. flexneri* and *V. cholerae* while leaf extract do not inhibit any test strain. Furthermore, the phytochemical study correlate with the study carried out by Deshmukh et al.[30]

Hooda et al.[32] reported presence of phytochemicals such as carbohydrate, protein, saponin, flavonoid, alkaloid and tannin in the root extracts of *Smilax zeylanica*. The present study finds presence of phytochemicals such as carbohydrate, saponin, flavonoid and alkaloid. Nonetheless, antibacterial activity is the primary information reported on ethanol extract of this plant.

Parekh and Chanda[33] studied antibacterial activity of *W. fruticosa* flowers and result found that methanol extract exhibited potential activity against *B. cereus*, *S. aureus*, *S. epidermidis*, *P. vulgaris*, *P. seudoalcaligenes* and *S. typhimurium*. Later, similar experiment carried out by Kumarswamy et al.[34] showed promising antibacterial activity against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *S. typhi*, *S. boydii*, *S. flexneri*, *S. sonnei* and *S. aureus*. Chougale et al.[35] also studied antibacterial activity of fraction of leaf extracts of *W. fruticosa* with promising



antibacterial activity against *B. subtilis*, *E. coli*, *S. aureus* and *P. aeruginosa*. Further, Bhattarai and Bhujul<sup>[36]</sup> tested both leaf and flower extracts of *W. fruticososa* and concluded that methanol extracts have antibacterial activity against *B. cereus*, *E. coli*, *P. aeruginosa*, *P. mirabilis*, *S. dysenteriae*, *S. typhimurium* and *S. aureus*. Finose and Devaki<sup>[37]</sup> investigated presence of phytochemicals viz. carbohydrate, amino acid, glycoside, saponin, flavonoid, alkaloid and tannin in *W. fruticososa*. Later, Gyawali *et al.*<sup>[38]</sup> establish presence of same phytoconstituents in addition with terpenoids.

The detail study and uses of these plants clearly indicate that the presence of these plant materials and their bactericidal activity are mainly responsible for protecting and preserving starter cultures since traditional system of fermentation normally operates in unhygienic condition which sometimes contaminates the system and cause toxication of drinks. But due to the presence of antimicrobial chemical principles of these plants or plant parts, they are able to continue such practice for generations without much decline in the characteristics of microorganisms involved in fermentation.

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## Conclusion

The study concludes that the plants used by tribals of Mayurbhanj District for the preparation of starter cultures are antimicrobials. Further study is required to evaluate the chemical constituents of these plants comparing with constituents of Bakhar.

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