Accepted Manuscript

Antimicrobial activity of select edible plants from Odisha, India against food-borne pathogens

Sujogya Kumar Panda, Yugal Kishore Mohanta, Laxmipriya Padhi, Walter Luyten

PII: S0023-6438(19)30576-6

DOI: <https://doi.org/10.1016/j.lwt.2019.06.013>

Reference: YFSTL 8246

To appear in: *LWT - Food Science and Technology*

Received Date: 30 October 2018

Revised Date: 17 March 2019

Accepted Date: 5 June 2019

Please cite this article as: Panda, S.K., Mohanta, Y.K., Padhi, L., Luyten, W., Antimicrobial activity of select edible plants from Odisha, India against food-borne pathogens, *LWT - Food Science and Technology* (2019), doi: <https://doi.org/10.1016/j.lwt.2019.06.013>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Title:

Antimicrobial activity of select edible plants from Odisha, India against food-borne pathogens

Author names:

Sujogya Kumar Panda^{*1,2,3}, Yugal Kishore Mohanta⁴, Laxmipriya Padhi^{2,3}, Walter Luyten¹

Affiliations:

¹ Department of Biology, KU Leuven, 3000 Leuven, Belgium

² Department of Zoology, North Orissa University, Baripada-757003, Odisha, India

³ Mayurbhanj Biological Research (MBR), Baripada-757002, Odisha, India

⁴ Department of Botany, North Orissa University, Baripada-757003, Odisha, India

Corresponding Author:

Sujogya Kumar Panda, Ph. D.

E-mail address: sujogya.panda@kuleuven.be

sujogyapanda@gmail.com

Tel. +32(0)16 373467; +32(0)16 330283 (Secretary)

Authors contributions:

Conceived and designed the experiments: Panda SK, Luyten W

Performed the experiments: Panda SK, Mohanta YK, Padhi L

Analyzed the data: Panda SK, Luyten W

Contributed reagents/materials/analysis tools: Panda SK, Luyten W

Contributed to the writing of the manuscript: Panda SK, Mohanta YK, Luyten W

pathogens

Abstract

The anti-microbial activity of crude extracts (hexane, acetone, ethanol, and aqueous) of 46 edible plants from Odisha, India, was studied using a broth microdilution assay against 8 common food-borne pathogens (Gram-positive: *Bacillus cereus* *Streptococcus faecalis*, *Listeria innocua*, *Micrococcus luteus*, and Gram-negative: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Shigella sonnei*). In total, 46 plant species belonging to 31 families were tested, from which 10 plants (22%) showed broad-spectrum *in vitro* antimicrobial activity (inhibiting at least two Gram-positive and Gram-negative species), while 28 (60%) exhibited narrow-spectrum activity against only Gram-positive -, and 2 (4%) against only Gram-negative bacteria. The Gram-positive pathogens were thus clearly more susceptible to most extracts compared to Gram-negative species. Selected active plant extracts were tested for their cytotoxicity against rhabdosarcoma (RD) and Vero cell lines. Selected extract(s) were further subjected to Thin Layer Chromatography (TLC) to determine their phytochemical profile; the extracts were largely distinguished by the presence of different phytoconstituents like phenols, flavonoids, tannins, saponins, steroids and terpenes. This preliminary antimicrobial screening showed that some edible Indian plants with antibacterial properties have potential as herbal preservatives in the food industry, and perhaps also as alternative therapeutic agents, particularly against food-borne bacterial diseases.

Keywords: Edible plants, Antimicrobial activity, Food-borne pathogens, Cytotoxicity, Thin Layer Chromatography, Odisha

27 Food spoilage due to microbial contamination is a major problem for consumers, industries
 28 and regulatory agencies (Penha et al., 2017). There are numerous food-borne pathogens that
 29 cause illness and occasionally even death. In the USA, approximately 48 million people are
 30 affected annually by a food-borne illness, with 3000 fatalities¹. To ensure microbial
 31 elimination and safety, the industry relies on the use of chemical preservatives,
 32 pasteurization, and other methods such as dehydration, irradiation, and high hydrostatic
 33 pressure (Gonzalez and Barret, 2010). New preservation treatments using photodynamic
 34 inactivation appear a promising strategy to eradicate all classes of microorganisms (Alves et
 35 al., 2015). “However, the application of this technique to the inactivation of microbes in food
 36 products is dependent on the availability of food-compatible photosensitizers that do not
 37 compromise food composition, appearance, taste and flavor” (Cunha et al., 2017).

38 Most traditional food preservation methods depend on the application of high temperature
 39 and pressure. The mild heat processing and modified-atmosphere packaging, adopted for
 40 preserving food products, are not sufficiently reliable for effective control of spoilage by
 41 pathogenic microorganisms. Moreover, low temperature storage of perishable foods cannot
 42 assure the safety and quality of food products (Negi, 2012; Tajkarimi, Ibrahim, Cliver,
 43 2010), and it costs a lot of energy. Finally, chemical preservatives alone cannot destroy all
 44 food pathogens or restrict microbial spoilage and are increasingly frowned upon by (at least
 45 some) consumers. Many consumers prefer to avoid chemical preservatives and are concerned
 46 about negative side effects. Chemical preservatives like nitrates, benzoates, sulphites,
 47 sorbates, parabens, formaldehyde, butylated hydroxytoluene or – hydroxyanisole, can cause
 48 serious health hazards such as hypersensitivity, allergy, asthma, hyperactivity, neurological
 49 damage or cancer. (Anand and Sati, 2013). Therefore, serious attention should be devoted to
 50 the safety of using chemical preservatives in the food industry (Shakiba, Kariminik, Parsia,

¹ <https://www.fda.gov/Food/FoodborneIllnessContaminants/FoodborneIllnessesNeedToKnow/default.htm>

2011; Tajkarimi, Ibrahim, Cliver, 2010). So, elimination of microbes from food without compromising the desirable properties of the product is still a challenge for the food industry. Research on controlling the spoilage and increasing the shelf life as well as the quality of food products, reveals the need for specific measures and technologies at each production step.

Also due to overuse of antibiotics, antibiotic-resistant (ABR) bacteria may reach humans via the food chain through consumption of contaminated food or food-derived products (Chang Wang, Regev-Yochay, Lipsitch, & Hanage, 2015; Rios & Recio, 2005). Typically, antibiotics used for treating humans are prohibited for use in other applications (veterinary, food, agriculture). Therefore, for public health and scientific reasons, strategic research should be expanded on natural antimicrobials as viable substitutes for chemically synthesized antimicrobials. Those alternatives could be safe, effective and acceptable natural preservatives. Natural plant antimicrobials such as plant extracts, essential oils and organic acids may offer considerable advantages as potential substitutes to safeguard our food safety (Hsouna et al., 2011; Negi, 2012; Takahashi, Kashimura, Koiso, Kuda, Kimura, 2013). For centuries, based on ethnomedicinal knowledge, edible plant extracts and essential oils have been traditionally used as folk medicine and as additives in food to extend the shelf life of products (Al-zoreky & Al-Taher, 2015). Moreover, the lower price of herbal medicine compared to conventional pharmaceuticals and some synthetic chemicals, encourages their cost-effective use for large-scale food preparation, which ultimately benefits also consumers (Shakiba, Kariminik, Parsia, 2011). The major advantages of using natural plant-based preservatives to replace synthetic chemical ones in food to control microbial contamination and growth, are: eliminating food pathogens to extend shelf life, reducing the emergence of ABR (not only in food pathogens), acting as better antioxidants to inhibit oxidation of e.g. lipids in meat products, and strengthening the immune system of the consumer by increasing immunoglobulin synthesis (Kim, Cho, Han, 2013; Tajkarimi, Ibrahim, Cliver, 2010).

77 Wild edible plants are crucial parts of plant biodiversity and their sustainable exploitation has
 78 become a valuable sustenance strategy and fall-back option for rural livelihood during
 79 periods of nutritional stress. Due to low food intake and limited access to food, food and
 80 nutritional security are essential matters in many areas around the world (Andersen, Thilsted,
 81 Nielsen, & Rangasamy, 2003; Mahapatra & Panda, 2012). Wild edible plants are accessible
 82 from common natural habitats and provide a source of food without cultivation or
 83 domestication (Pegu et al., 2013).

84 The Odisha state of India is known as a genetic paradise for its rich diversity in plant genetic
 85 resources for many indigenous food crops and forest plant species (Mishra, Panda, Sahu,
 86 2012; Panda & Luyten, 2018). The resident tribal communities are mainly dependent upon
 87 the locally available resources, and the edible plants are distinctly reflected in their traditional
 88 food habits. Moreover, these communities possess ample knowledge on wild edible plants,
 89 including their use for treating common ailments such as diarrhoea, dysentery, cold, malaria
 90 fever, vomiting, headache, etc. (Swain & Mohapatra, 2013).

91 In this study, therefore, we tested 46 different edible plants, selected based on their uses
 92 described in the scientific literature and in traditional knowledge, as remedies against eight
 93 major food-borne pathogens. The findings of the current study could pave the way for further
 94 detailed studies on Indian traditional medicinal plants as edible resources, suitable as natural
 95 preservatives for the food-processing industry. Furthermore, we tested active extracts
 96 (showing antibacterial activity with MIC₅₀ values against food-borne pathogens <1000
 97 µg/mL) for cytotoxicity against both RD - and Vero cell lines. We also briefly consider their
 98 broader potential as antimicrobial therapeutics.

99

100 **2. Materials and methods**

101 *2.1 Plant materials*

Forty-six edible plant species were collected for evaluation of their antimicrobial activity based on their uses in the scientific literature and in traditional knowledge. The different parts (leaf, bark, root, rhizome, flower, fruit or seed) of these plants were collected in the wild and a few were purchased from regional markets (Odisha, India). They were all authenticated by an eminent plant taxonomist of the Department of Botany, North Orissa University, Baripada, where voucher specimens are deposited. None of them are endangered or protected species. The information on these plants with their official taxonomic name (according to the plant list: <http://www.theplantlist.org>), edible use, used part(s), and voucher numbers are shown in Table 1.

2.2 Extraction preparation

The plant parts (e.g. leaves, bark, seeds or fruits) were dried separately in the shade at ambient temperature (approximately 35°C). They were ground by a mechanical grinder and filtered through a 40-mesh sieve to yield a fine powder. All the materials were stored at 4°C completely dried so as not to permit growth of fungi, molds, bacteria or other microorganisms (Panda et al., 2016). For the small-scale extraction, we followed the same procedure as in our previous study (Panda et al., 2017). The dry residue was dissolved in DMSO to a final stock concentration of 20 mg/mL. The samples were stored at 4°C until further testing.

2.3 Antimicrobial activity

2.3.1 Microorganisms

The bacterial strains used in this study were: *Escherichia coli* (Top 10 Invitrogen), *Pseudomonas aeruginosa* (PAO1), *Bacillus cereus* (DPMB 1), *Streptococcus faecalis* (DPMB 4), *Listeria innocua* (LMG 11387), *Micrococcus luteus* (DPMB 3), *Salmonella enterica* subsp. *enterica* (ATCC 13076) and *Shigella sonnei* (LMG 10473).

2.3.2 Preparation of inoculum

Stock cultures were maintained at -80°C in 25% glycerol. Colonies were inoculated on Mueller-Hinton (MH) agar plates, and after overnight growth at 37°C, plates were sealed

128 with parafilm and stored in a cold room (4°C). A single colony of each microorganism was
 129 transferred into sterile MH broth for *E. coli*, *P. aeruginosa*, *B. cereus*, *L. innocua*, *S. sonnei*,
 130 *M. luteus*, and into tryptic soy (TS) broth for *S. faecalis* and *S. enterica*. The reaction tubes
 131 were incubated overnight while shaking at 200 rpm and 37°C.

132 2.3.4 Antimicrobial assay (broth microdilution method) and determination of 133 minimum inhibitory concentration (MIC₅₀)

134 Antimicrobial activity was assessed as described previously (Panda, Das, Leyssen, Neyts,
 135 Luyten, 2018) using a 96-well microdilution method. A two-fold serial dilution series (up to
 136 32-fold) using DMSO for organic extracts and water for aqueous extracts from a stock
 137 solution of 20 mg/mL was prepared in a separate 96-well conical bottom (V) microplate. Ten
 138 µL of the test sample was transferred into the wells of a 96-well plate, as well as the positive
 139 control (ciprofloxacin, stock 100 µg/mL) and blank (solvent) controls (DMSO and water).
 140 Each well of a microdilution plate was inoculated with 190 µL of the diluted standardized
 141 inoculum using MH or TS broth (OD = 0.003 at 620 nm). Control wells were prepared with
 142 190 µL MH or TS broth, plus 10 µL extract to correct for any absorption due to extract
 143 components. The relative inhibition (%) of the test sample was calculated by dividing (the
 144 OD value of the test sample minus that of the non-inoculated extract control) by the average
 145 OD of the solvent control, and multiplying by 100. Data from dose-response experiments
 146 were represented as the percentage of inhibition, and analysed with Prism™ (GraphPad
 147 Prism 5.0 Software Inc., San Diego, CA). The MIC₅₀ for each growth condition was
 148 calculated by fitting the data to a non-linear least-squares sigmoid regression curve, fixing the
 149 top and bottom of the curve at 100 and 0 percent, respectively. The MIC₅₀ corresponds to the
 150 concentration that would yield an inhibition of 50%.

151 2.3.5 Determination of *in vitro* cytotoxicity

152 The cytotoxic effect of selected extract(s) was quantified using a colorimetric readout with 3-
 153 (4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-

154 tetrazolium/phenazine methosulfate (MTS/PMS method). Briefly, the rhabdomyosarcoma (RD)
 155 and Vero cells were seeded in 96-well plates at a density of 2×10^3 cells/well and
 156 2.5×10^3 cells/well, respectively, in 100 μ L assay medium (MEM Rega 3 medium with 2%
 157 fetal bovine serum, 1% 200 mM L-glutamine and 1% 7.5% NaHCO_3). Following attachment
 158 and 24 h incubation, the cells were treated with a serial dilution of the extract(s), followed by
 159 the addition of 100 μ L assay medium, and incubated for 72 h. To calculate CC_{50} a dose-
 160 response curve is plotted, and the concentration that causes death to 50% of viable cells is
 161 derived by interpolation (as explained above under 2.3.4). The final, maximal DMSO
 162 concentration that was reached in the assay wells with the highest sample input (1%) was
 163 well tolerated by the cells.

164 2.3.6. Thin layer chromatography (TLC) and phytochemical analysis

165 Phytochemical analysis by means of TLC was carried out for plants with active extracts,
 166 following the method of Wagner and Bladt (2001) with slight modification (Panda, Das,
 167 Leyssen, Neyts, Luyten, 2018). The dried extracts were reconstituted in acetone/ethanol to a
 168 concentration of 5 mg/mL. The TLC plates were developed with ethyl
 169 acetate:dichloromethane:formic acid (8:1:1, v/v); and methanol: dichloromethane (9:1, v/v) at
 170 ambient temperature (approximately 20°C), and dried in an oven at 100°C for 5 minutes to
 171 evaporate the solvent. One set of plates was used for visualization of the spots by spraying
 172 with 5% sulphuric acid in ethanol, followed by heating at 100°C for 5 minutes, while the
 173 other set was visualized under UV. Detection of the phytochemicals was conducted by
 174 comparison of R_f values and spot colours with literature data.

175

176 3. Results

177 The majority of the plants are consumed as fruits (23 plants, 40%), followed by leaves (19
 178 plants, 33%). The remainder of the plants are consumed in the form of bark, root, rhizome,
 179 flower or seed (Table 1). For the present study, 46 plant species were extracted with four

different solvents, and their yields are presented as supplementary material 1. Among all plant parts, leaves are showing most often antimicrobial activity, while the contribution of other plant parts was insignificant (Figure 1). The collected plants represent 30 families, of which the most common ones are Leguminosae (5 species), followed by Anacardiaceae, Convolvulaceae and Phyllanthaceae (3 species), followed by Combretaceae, Compositae, Dilleniaceae, Lythraceae, Rutaceae and Sapotaceae (2 species). Interestingly, all the test plants from the Leguminosae family such as *Bauhinia semla*, *Butea monosperma*, *Clitoria ternatea*, *Pithecellobium dulce* and *Tamarindus indica* showed antimicrobial activity (Table 2).

In the present study, many extracts significantly inhibited one or more of the tested microorganisms (Table 2, list of 25 plants that show inhibition >80% at a concentration of 1 mg/mL, Table 3, list of 25 plants that show inhibition >50% at a concentration of 1 mg/mL). Among the Gram-positives, the most sensitive strains were *M. luteus* (73% extracts are active) and *L. innocua* (96% extracts are active) (Figure 2). A few notable plant extracts with MIC₅₀ values < 200 µg/mL against *L. innocua* are the ethanol extract of *Limonia acidissima*, the water extracts of *Woodfordia fruticosa*, *Ocimum sanctum*, *Mimusops elengi*, *Semecarpus anacardium* and *Terminalia arjuna*, the acetone extract of *Bauhinia semla*, *Curculigo orchioides*, *Gardenia latifolia*, *Syzygium nervosum* and *Woodfordia fruticosa* (Table 4). Similarly, extracts of select plants such as *Argyreia speciosa*, *Bauhinia racemosa*, *Casearia graveolens*, *Careya arborea*, *Madhuca longifolia*, *Rubus ellipticus*, *Symplocos cochinchinensis*, *Terminalia arjuna*, and *Tinospora cordifolia*, were also able to inhibit both *B. cereus* and *S. faecalis* (Supplementary material 2). Some plants are noticed to have a narrow activity spectrum, inhibiting only particular bacterial species (Supplementary material 2, Table 2).

Compared to Gram-positives, fewer plants show activity towards Gram-negatives (Supplementary material 3, inhibition >50% at a concentration of 1 mg/mL). Only a few

plants such as *Aegle marmelos*, *Argyreia speciosa*, *Bauhinia semla*, *Boerhavia diffusa*, *Glochidion lanceolarium*, *Mimusops elengi*, *Madhuca longifolia*, *Ocimum sanctum*, *Syzygium nervosum*, *Terminalia arjuna* and *Woodfordia fruticosa*, show narrow-spectrum activity: inhibition only against *E. coli* or *P. aeruginosa* (Table 3). However, plants such as *Semecarpus anacardium*, *Tinospora cordifolia* and *Gardenia latifolia*, inhibit both *E. coli* (MIC₅₀ –593, 645 and 380 µg/mL, respectively) and *P. aeruginosa* (MIC₅₀ –432, 556 and 98 µg/mL, respectively) (Table 4). *Salmonella enterica* was found to be the most resistant micro-organism and was inhibited by only three plants: *Semecarpus anacardium*, *Punica granatum* and *Curcuma amada*. Plants such as *Argyreia speciosa*, *Bauhinia racemosa*, *Bauhinia semla*, *Casearia graveolens*, *Careya arborea*, and *Dillenia pentagyna* are effective against *S. sonnei*, with MIC₅₀ values ranging from 356 to 970 µg/mL (Table 4).

Extracts prepared using the four different solvents have dissimilar antimicrobial activity profiles. Ethanol (42%) and acetone (34%) extracts were found to be more effective compared to aqueous (14%) and hexane (10%) extracts (Figure 2). Select active plant extracts with MIC₅₀ values <1000 µg/mL were studied for cytotoxicity (CC₅₀) against two cell lines: RD and Vero cells (Table 5). We found that 14 plant extracts (36%) showed cytotoxicity against both RD and Vero cell lines, whereas only 7 plant extracts (18%) did not show any cytotoxicity. Moreover, 23% of plant extracts show toxicity for the Vero cell line and 20% for RD cells. Most plant extracts have CC₅₀ values >100 µg/mL against Vero cells. Plants such as *Aegle marmelos*, *Bauhinia semla*, *Dillenia pentagyna*, *Gardenia latifolia*, *Glochidion lanceolarium*, *Gymnema sylvestre*, *Limonia acidissima*, *Madhuca longifolia*, *Piper longum*, *Protium serratum*, *Rubus ellipticus*, *Semecarpus anacardium*, *Syzygium nervosum*, *Terminalia chebula*, *Tinospora cordifolia* and *Woodfordia fruticosa* were toxic at concentrations <50 µg/mL (Table 5).

To develop fingerprints, TLC was used for at least one extract of each active plant. Acetone and ethanol extracts were subjected to TLC with methanol:dichloromethane (9:1, v/v) in

order to separate the phytoconstituents (supplementary material 5). Different absorbing bands were observed under UV light (254 nm and 360 nm). Mostly spots of pink, yellow, green, brown, purple, violet blue and dark brown colours were observed on TLC plates under UV, which indicates the presence of flavonoids, polyphenols, sterols and glycosides, terpenoids, alkaloids, etc. The pink and light-yellow bands confirmed the presence of flavonoids, violet blue-coloured spots indicate saponins, purple and bluish-violet colours indicate terpenoids, while the development of a dark green colour after 5% sulphuric acid indicates the presence of steroids (Debenedetti, 2009). The TLC data were clustered and heat maps were prepared to distinguish between extracts for different phytochemicals and presented in Figure 4. Most of the plants contain flavonoids. Plants such as *Bauhinia semla*, *Clitoria ternatea*, *Centella asiatica*, *Glochidion lanceolarium* and *Punica granatum* are placed in one cluster with the presence of only flavonoids. Two plants *Gymnema sylvestre* and *Terminalia arjuna* are placed in another cluster due to the presence of alkaloids. *Terminalia chebula* was found to contain alkaloids along with flavonoids and terpenoids. The group of *Ardisia solanacea* and *Mimusops elengi* shows the presence of flavonoids and saponins. Moreover, *Enhydra fluctuans* (*Meyera fluctuans*) and *Lannea coromandelica* contain flavonoids and saponins but differ from other clusters by the presence of additional phytochemicals such as polyphenols and tannins, respectively. *Semecarpus anacardium* forms a separate cluster with the presence of flavonoids, sterols and glycosides.

251

252 4. Discussion

253 The extraction of biologically active compounds from plant material is largely dependent on
 254 the type of the solvent used in the extraction procedure, as well as the temperature and
 255 duration of the extraction. In our study, four different solvents were used for extraction:
 256 hexane, ethanol, acetone, and water. Antimicrobial activity is seen less in the hexane extracts,
 257 while almost all other solvents viz. ethanol, acetone and water have yielded significant

antimicrobial activity against both Gram-positive and Gram-negative bacteria (supplementary material 2, 3 and 4). Among the solvents used, ethanol appears the best for extracting antimicrobial compounds, followed by acetone. Ethanol is often recommended for food and pharmaceutical processing because of its safety and affordability (Guo et al., 2001). Several of our earlier studies have reported significant bioactivity of plant extracts in alcohol, which dissolves both relatively polar as well as more nonpolar plant compounds (Kerkoub et al., 2018; Panda, 2014; Panda et al., 2016; 2017). Others have reported similar finding and have rated methanol and ethanol as the best solvents for the extraction of antimicrobial compounds from plants, followed by acetone and water (Shan, Cai, Brooks, Corke, 2007; Sharma, Aneja, Kasera, & Aneja, 2012; Sunilson et al., 2009; Weerakkody, Caffin, Turner, Dykes, 2010).

Of the 46 plants screened, 13 (29%) showed activity in at least one extract against *E. coli*, 15 (33%) against *Bacillus cereus*, 16 (36%) against *Streptococcus faecalis*, 43 (96%) against *Listeria innocua*, 33 (73%) against *Micrococcus luteus*, 14 (31%) against *Pseudomonas aeruginosa*, 7 (16%) against *Salmonella enterica*, and 9 (20%) against *Shigella sonnei* (Figure 3). So, most extracts were able to inhibit Gram-positive food-borne pathogens, whereas fewer inhibit Gram-negative food-borne pathogens (Figure 1 and 3). Surprisingly, several plants previously collected from Odisha and reported as having antimicrobial activity against similar strains tested in the present study were not able to inhibit at a concentration of 1 mg/mL with extracts prepared in any of the four solvents. The list includes *Butea monosperma* against *E. coli* (Dubey et al., 2012; Sahu & Padhy, 2013), *Bridelia retusa* against *E. coli* and *P. aeruginosa* (Mishra & Padhy, 2013), *Boerhavia diffusa* against *P. aeruginosa* (Mishra et al., 2017), *Curcuma amada* against *P. aeruginosa* (Rath & Padhy, 2014), *Cinnamomum zeylanicum* against *E. coli* and *P. aeruginosa* (Rath & Padhy, 2014), *Mimusops elengi* against *E. coli* (Mishra & Padhy, 2013), *Punica granatum* against *E. coli* and *P. aeruginosa* (Mishra et al., 2017). Additionally, plants such as *Argyreia speciosa* and *Woodfordia fruticosa* previously not found to be active by

284 Dubey and coworkers (2012) were found to be effective against *P. aeruginosa* and *E. coli*
 285 respectively.

286 This could be due to differences in the plant parts used, the extraction procedure or the
 287 bioassay (method or microbial strain).

288 Antimicrobial agents usually target some cellular mechanism involved in cell metabolism
 289 (Sondi & Salopek-Sondi, 2004). Major metabolic pathways involve DNA replication, protein
 290 synthesis, ATP production, intermediate metabolism, cell wall synthesis, etc., and the
 291 antimicrobial resistance starts when such mechanisms or processes are inactive or not
 292 influenced any more by antimicrobial agents. Our results show that Gram-negative bacteria
 293 are less susceptible than Gram-positive bacteria, which may be due to a lack of permeability
 294 of the lipopolysaccharide outer membrane (Figure 1).

295 The leaves were by far the most common plant part that showed bioactivity. This is fortunate
 296 since it is often the most abundant part, that is moreover available for most of the growing
 297 season (as opposed to flowers and (ripe) fruit) and can be easily and sustainably harvested (as
 298 opposed to, for instance, rhizomes). The most active plants extract (with MIC₅₀ values against
 299 food-borne pathogens <1000 µg/mL) were also evaluated for cytotoxicity (CC₅₀) against two
 300 cell types: the RD - and Vero cell line (Table 5). When extracts would be added to food for
 301 preservative purposes, they will be ingested and may exert effects in the gastrointestinal tract.
 302 Given the long-standing use as edible plant, such oral administration is expected to be safe,
 303 and extracts could be used as preservatives in the food industry, as well as for some
 304 gastrointestinal problems (e.g. in case of diarrhoea).

305 However, some compounds may be absorbed by the gastrointestinal tract, and become
 306 available systemically, opening up additional therapeutic possibilities (as well as potential
 307 toxicities). Therefore, we tested the extracts for cytotoxic effects on two mammalian cell
 308 lines. Extracts from 14 plant such as *Aegle marmelos*, *Boerhavia diffusa*, *Dillenia pentagyna*,
 309 *Emblica officinalis*, *Meyera fluctuans*, *Gardenia latifolia*, *Gymnema sylvestre*,

310 *Limonia acidissima*, *Mimusops elengi*, *Piper longum*, *Protium serratum*, *Rubus ellipticus*,
 311 *Semecarpus anacardium*, *Syzygium nervosum*, *Terminalia chebula*, *Tinospora cordifolia* and
 312 *Woodfordia fruticosa* showed cytotoxicity against either or both cell lines at concentrations
 313 <100 µg/mL. However, they should not necessarily be discarded from further study as these
 314 are crude extracts typically containing hundreds of compounds, and the antimicrobial
 315 compound(s) in the extract may not be the ones causing cytotoxicity. Therefore, all these
 316 plants may still have potential for discovering new antibiotic drug candidates. Extract(s) with
 317 strong antimicrobial activity combined with low cytotoxicity, from plants such as *Argyrea*
 318 *speciosa*, *Bauhinia racemosa*, *Bauhinia semla*, *Careya arborea*,
 319 *Casearia graveolens*, *Diospyros malabarica*, *Gardenia latifolia*, *Madhuca longifolia*,
 320 *Ocimum sanctum* and *Symplocos cochinchinensis* could be considered a promising sources of
 321 new drug candidates in addition to their use as preservatives in the food industry.

322 Based on the colour on TLC plates, the secondary metabolites were differentiated and R_f
 323 values were calculated (supplementary material 5). Compared to other chromatographic
 324 techniques, TLC is a simple, economical, rapid and flexible technique allowing sensitive
 325 parallel processing of many samples on one plate. Therefore, we used TLC as a “fingerprint”
 326 method to characterize our plant extracts. Because we use crude extracts from diverse
 327 medicinal plants, most are very complex matrices, and a simple TLC separation cannot
 328 resolve all components with a single mobile phase. Many extracts how similar (though
 329 distinct) fingerprints (observed colour as well as R_f), using the same mobile phase, signifying
 330 the presence of similar chemical classes. The results from the TLC study suggest that there
 331 are several groups of phytochemical constituents, but which of these constitute the bioactive
 332 compounds responsible for the antimicrobial activity needs to be elucidated.

333 Many compounds from plant origin are recognised as safe, and exempted from the category
 334 of food additives (Negi, 2012). Botanical edibles are also gaining importance for use in
 335 ethnomedicine or herbal medicine by both the general public and health professionals, due to

the relatively lower incidence of both adverse effects and pathogens acquiring resistance against antibiotics (Newell et al., 2010). The present paper paves the way for use in the food-processing industry of edible plants based on traditional knowledge. However, further detailed study is required to find novel drug candidates.

340

341 **5. Conclusion**

The broth microdilution assay demonstrated significant antimicrobial activity against food-borne pathogens in most of the 46 edible plant species from India that we tested. Wild edible plants such as *Argyreia speciosa*, *Bauhinia racemosa*, *Bauhinia semla*, *Careya arborea*, *Casearia graveolens*, *Diospyros malabarica*, *Gardenia latifolia*, *Madhuca longifolia*, *Ocimum sanctum* and *Symplocos cochinchinensis* could be considered a promising source of preservatives in the food industry as well as perhaps new drug candidates. Further in-depth research needs to include *in vivo* tests to determine the effectiveness, stability and impact of the studied extracts (and their bioactive compounds) on controlling food pathogens, and to evaluate their potential as preservatives to prolong the shelf life of food.

351

352 **6. Acknowledgements**

The authors wish to thank the authorities of North Orissa University for providing necessary facilities for collecting and identifying plant materials. We are also thankful to Dr. Anil K. Biswal, Dr. Akshaya K. Bastia, Dr. Hemant K. Sahu, Dr. Gunanidhi Sahoo (North Orissa University) for the extraction of plant samples and expert technical help during experiments. Thanks also to Purity Ngina Kipanga and Pranav Godse (KU Leuven), for their technical help in preparing and extracting plant materials, and for help with TLC photography. The author SKP is also grateful for receipt of a postdoc fellowship from EMINTE (Erasmus Mundus, EU; <http://eminte.eu/>). WL largely supported himself.

361

7. Conflicts of Interest

No potential conflict of interest was reported by the authors.

8. References

- Alves, E., Faustino, M. A. F., Neves, M. G. P. M. S., Cunha, A., Nadais, H., & Almeida, A. (2015). Potential applications of porphyrins in photodynamic inactivation beyond the medical scope. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, 22, 34-57. <https://doi.org/10.1016/j.jphotochemrev.2014.09.003>
- Al-zoreky, N. S., & Al-Taher, A. Y. (2015). Antibacterial activity of spathe from *Phoenix dactylifera* L. against some food-borne pathogens. *Industrial Crops and Products*, 65, 241–246. <https://doi.org/10.1016/j.indcrop.2014.12.014>
- Anand, S. P., & Sati, N. (2013). Artificial preservatives and their harmful effects: looking toward nature for safer alternatives. *International Journal of Pharmaceutical Sciences and Research*, 4(7), 2496–2501. doi: 10.13040/IJPSR.0975-8232.4(7).24960-01
- Andersen, L., Thilsted, S., Nielsen, B., & Rangasamy, S. (2003). Food and nutrient intakes among pregnant women in rural Tamil Nadu, South India. *Public Health Nutrition* 6, 131–137. <https://doi.org/10.1079/PHN2002367>
- Bandana, P., & Debabrata, P. (2015). Wild edible plant diversity and its ethno-medicinal use by indigenous tribes of Koraput, Odisha, India. *Research Journal of Agriculture and Forestry Sciences*, 3, 1–10.
- Chang, Q., Wang, W., Regev-Yochay G., Lipsitch M., & Hanage, W. P. (2015). Antibiotics in agriculture and the risk to human health: how worried should we be? *Evolutionary Applications*, 8, 240–245. doi: 10.1111/eva.12185
- Cunha, A., Couceiro, J., Bonifácio, D., Martins, C., Almeida, A., Neves, M. G. P. M. S., Faustino, M. A. F., & Saraiva, J. A., (2017). Inactivation of pathogenic bacteria in food matrices: high pressure processing, photodynamic inactivation and pressure-assisted

- 388 photodynamic inactivation. In: *IOP Conference Series: Earth and Environmental*
 389 *Science* (85, 1, p. 012016). IOP Publishing. doi :10.1088/1755-1315/85/1/012016
- 390 Debenedetti, S. L. (2009). TLC and PC. In Sampietro, D. A., Catalan, C. A. N., Vattuone, M.
 391 A. (Eds), *Isolation, identification and characterization of allelochemicals* (pp. 103-133);
 392 CRC Press.
- 393 Dubey, D., Sahu, M. C., Rath, S., Paty, B. P., Debata, N. K., & Padhy, R. N. (2012).
 394 Antimicrobial activity of medicinal plants used by aborigines of Kalahandi, Orissa, India
 395 against multidrug resistant bacteria. *Asian Pacific Journal of Tropical*
 396 *Biomedicine*, 2(2), S846-S854. doi: 10.1016/S2221-1691(12)60322-0
- 397 Gonzalez, M., & Barret, D. M. (2010). Thermal, high pressure, and electric field processing
 398 effects on plant cell membrane integrity and relevance to fruit and vegetable quality.
 399 *Journal of Food Science*, 75, 121-130. doi: 10.1111/j.1750-3841.2010.01763.x
- 400 Guo, Z., Jin Q, Fan, G., Duan, Y., Qin, C., & Wen, M. (2001). Microwave-assisted extraction
 401 of effective constituents from a Chinese herbal medicine *Radix*
 402 *puerariae*. *Analytica Chimica Acta*, 436, 41–47. [https://doi.org/10.1016/S0003-](https://doi.org/10.1016/S0003-2670(01)00900-X)
 403 2670(01)00900-X
- 404 Hsouna, A., Trigui, M., Mansour, R., Jarraya, R., Damak, M., & Jaoua, S. (2011). Chemical
 405 composition: cytotoxicity effect and antimicrobial activity of *Ceratonia siliqua* essential
 406 oil with preservative effects against *Listeria* inoculated in minced beef meat.
 407 *International Journal of Food Microbiology*, 148, 66–72. doi:
 408 10.1016/j.ijfoodmicro.2011.04.028
- 409 Kerkoub, N., Panda, S. K., Yang, M-R, Lu, J-G, Jiang, Z-H, Nasri, H., & Luyten, W. (2018).
 410 Bioassay-guided isolation of anti-candida biofilm compounds from methanol extract of
 411 the aerial parts of *Salvia officinalis* (Annaba, Algeria). *Frontiers Pharmacology*, 9,1418.
 412 doi: 10.3389/fphar.2018.01418
- 413 Kim, S., Cho, A., & Han, J. (2013). Antioxidant and antimicrobial activity of leafy green

- 414 vegetable extracts and their applications to meat product preservation. *Food Control*, 29,
 415 112–120. <https://doi.org/10.1016/j.foodcont.2012.05.060>
- 416 Mahapatra, A. K., & Panda, P. C. (2012). Wild edible fruit diversity and its significance in
 417 the livelihood of indigenous tribals: Evidence from eastern India. *Food Security*, 4(2),
 418 219–234. <https://doi.org/10.1007/s12571-012-0186-z>
- 419 Mishra, M. K., Panda, A., & Sahu, D. (2012). Survey of useful wetland plants of South
 420 Odisha, India. *Indian Journal of Traditional Knowledge*, 11(4), 658–666.
 421 <http://hdl.handle.net/123456789/14963>
- 422 Misra, S., & Misra, M. K. (2013). Leafy vegetable plants of South Odisha, India.
 423 *International Journal of Agricultural and Food Science*, 3, 131–137.
- 424 Mishra, M. P., & Padhy, R. N. (2013). In vitro antibacterial efficacy of 21 Indian timber-
 425 yielding plants against multidrug-resistant bacteria causing urinary tract
 426 infection. *Osong Public Health and Research Perspectives*, 4(6), 347–357.
 427 <http://dx.doi.org/10.1016/j.phrp.2013.10.007>
- 428 Mishra, M. P., Rath, S., Swain, S. S., Ghosh, G., Das, D., & Padhy, R. N. (2017). In vitro
 429 antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing
 430 MDR bacteria. *Journal of King Saud University-Science*, 29, 84–95.
 431 <http://dx.doi.org/10.1016/j.jksus.2015.05.007>
- 432 Misra, S., & Misra, M. K. (2016). Ethnobotanical and nutritional evaluation of some edible
 433 fruit plants of Southern Odisha, India. *International Journal of Advances in Agricultural*
 434 *Science and Technology*, 3, 1–30.
- 435 Negi, P. (2012). Plant extracts for the control of bacterial growth: efficacy stability and safety
 436 issues for food application. *International Journal of Food Microbiology*, 156, 7–17.
 437 <https://doi.org/10.1016/j.ijfoodmicro.2012.03.006>
- 438 Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H.,
 439 Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F., der Giessen, J. van, & Kruse, H.,

- (2010). Food-borne diseases - The challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology*, 139, S3–S15. <https://doi.org/10.1016/j.ijfoodmicro.2010.01.021>
- Panda, S. K. (2014). Ethno-medicinal uses and screening of plants for antibacterial activity from Similipal Biosphere Reserve, Odisha, India. *Journal of Ethnopharmacology*, 151, 158–175. doi:10.1016/j.jep.2013.10.004
- Panda, P., Das, D., Dash, P., & Ghosh, G. (2015). Therapeutic potential of *Bauhinia racemosa* - A mini review. *International Journal of Pharmaceutical Sciences Review and Research*, 28, 169–179.
- Panda, S. K., Mohanta, Y. K., Padhi, L., Park, Y.-H., Mohanta, T. K., & Bae, H. (2016). Large scale screening of ethnomedicinal plants for identification of potential antibacterial compounds. *Molecules*, 21(3). <https://doi.org/10.3390/molecules21030293>
- Panda, S. K., Padhi, L., Leyssen, P., Liu, M., Neyts, J., & Luyten, W. (2017). Antimicrobial, anthelmintic and antiviral activity of plants traditionally used for treating infectious disease in the Similipal Biosphere Reserve, Odisha, India. *Frontiers in Pharmacology*, 8, 658. doi: 10.3389/fphar.2017.00658
- Panda, S. K., & Luyten, W. (2018). Antiparasitic activity in Asteraceae with special attention to ethnobotanical use by the tribes of Odisha, India. *Parasite*, 25, 10. doi: 10.1051/parasite/2018008
- Panda, S. K., Das, R., Leyssen, P., Neyts, J., & Luyten, W. (2018). Assessing medicinal plants traditionally used in the Chirang Reserve Forest, Northeast India for antimicrobial activity. *Journal of Ethnopharmacology*, 225, 220–233. doi.org/10.1016/j.jep.2018.07.011
- Pegu, R., Gogoi, J., Tamuli, A. K., & Teron, R. (2013). Ethnobotanical study of wild edible plants in Poba Reserved Forest, Assam, India: Multiple functions and implications for conservation. *Research Journal of Agriculture and Forestry Sciences*, 1(3), 1–10.

- 466 Penha, C. B., Bonin, E., da Silva, A. F., Hioka, N., Zangueta, E. B., Nakamura, T. U., de
 467 Abreu Filho, B. A., Campanerut-Sá, P. A. Z., & Mikcha, J. M. G. (2017). Photodynamic
 468 inactivation of foodborne and food spoilage bacteria by curcumin. *LWT-Food Science
 469 and Technology*, 76, 198–202. <https://doi.org/10.1016/j.lwt.2016.07.037>
- 470 Rath, S., & Padhy, R. N. (2014). Monitoring in vitro antibacterial efficacy of 26 Indian spices
 471 against multidrug resistant urinary tract infecting bacteria. *Integrative Medicine
 472 Research*, 3(3), 133–141. <http://dx.doi.org/10.1016/j.imr.2014.04.002>
- 473 Rios, J., & Recio, M. (2005). Medicinal plants and antimicrobial activity. *Journal of
 474 Ethnopharmacology*, 100, 80–84. <https://doi.org/10.1016/j.jep.2005.04.025>
- 475 Sahu, M. C., & Padhy, R. N. (2013). *In vitro* antibacterial potency of *Butea*
 476 *monosperma* Lam. against 12 clinically isolated multidrug resistant bacteria. *Asian
 477 Pacific Journal of Tropical Disease*, 3(3), 217–226. doi:10.1016/S2222-1808(13)60044-
 478 4
- 479 Shakiba, M., Kariminik, A., & Parsia, P. (2011). Antimicrobial activity of different parts of
 480 *Phoenix dactylifera*. *International Journal of Molecular and Clinical Microbiology*, 1,
 481 107–111. <http://dx.doi.org/10.4314/bajopas.v10i1.7S>
- 482 Shan, B., Cai, Y.-Z., Brooks, J., & Corke, A. (2007). The in vitro antibacterial activity of
 483 dietary spice and medicinal herb extracts. *International Journal of Food Microbiology*,
 484 117, 112–119. doi: 10.1016/j.ijfoodmicro.2007.03.003
- 485 Sharma, C., Aneja, K., Kasera, R., & Aneja, R. (2012). Antimicrobial potential of *Terminalia*
 486 *chebula* Retz. Fruit extracts against ear pathogens. *World Journal of
 487 Otorhinolaryngology*, 2, 8–13. doi: 10.5319/wjo.v2.i2.8
- 488 Sinha, R., Lakra, V., (2005). Wild tribal food plants of Orissa. *Indian Journal of Traditional
 489 Knowledge*, 4, 246–252. <http://hdl.handle.net/123456789/8510>
- 490 SonDI, I., & Salopek-Sondi, B., (2004). Silver nanoparticles as antimicrobial agent: a case
 491 study on *E. coli* as a model for Gram-negative bacteria. *Journal of Colloid and Interface*

- 492 *Science*, 275, 177–182. <https://doi.org/10.1016/j.jcis.2004.02.012>
- 493 Sunilson, J., Suraj, R., Rejitha, G., Anandarajagopal, K., Kumari, A., & Promwichit, P.
 494 (2009). *In vitro* antimicrobial evaluation of *Zingiber officinale*, *Curcuma longa* and
 495 *Alpinia galanga* extracts as natural food preservatives. *Journal of Food Technology*,
 496 4(5), 192–200. doi: 10.3923/ajft.2009.192.200
- 497 Swain, S., & Mohapatra, G. C. (2013). Multiple usages of forest trees by the tribes of
 498 Kalahandi District, Orissa, India. *International Journal of Biodiversity and*
 499 *Conservation*, 5(6), 333–341. doi: 10.5897/IJBC11.129
- 500 Tajkarimi, M. M., Ibrahim, S. A., & Cliver, D. O. (2010). Antimicrobial herb and spice
 501 compounds in food. *Food Control*, 21(9), 1199–1218.
 502 <https://doi.org/10.1016/j.foodcont.2010.02.003>
- 503 Takahashi, H., Kashimura, M., Koiso, H., Kuda, T., & Kimura, B. (2013). Use of ferulic acid
 504 as a novel candidate of growth inhibiting agent against *Listeria monocytogenes* in ready-
 505 to-eat food. *Food Control*, 33, 244–248. <https://doi.org/10.1016/j.foodcont.2013.03.013>
- 506 Ved, D. K., & Goraya, G. S. (2007). Demand and supply of medicinal plants in India.
 507 NMPB, New Delhi & FRLHT, Bangalore, India.
- 508 Wagner, H., & Bladt, S. (2001). Plant drug analyses: A thin layer chromatography analysis.
 509 (2nd ed.). Berlin, Springer-Verlag.
- 510 Weerakkody, N., Caffin, N., Turner, M., & Dykes, G. (2010). *In vitro* antimicrobial activity
 511 of less-utilized spice and herb extracts against selected food-borne bacteria. *Food*
 512 *Control*, 21(10), 1408–1414. <https://doi.org/10.1016/j.foodcont.2010.04.014>
- 513
- 514 Appendix A. Supplementary data 1
- 515 Appendix B. Supplementary data 2
- 516 Appendix C. Supplementary data 3
- 517 Appendix D. Supplementary data 4

519

520 **List of tables**521 **Table-1:** List of select edible plants of Odisha, and their uses522 **Table-2:** Antimicrobial activity of 25 plants showing strong inhibition (>80% at a
523 concentration of 1 mg/mL) against Gram-positive food-borne pathogens524 **Table-3:** Antimicrobial activity of 25 plants showing strong inhibition (>50% at a
525 concentration of 1 mg/mL) against Gram-negative food-borne pathogens526 **Table 4:** MIC₅₀ of plant extracts against select food-borne pathogens527 **Table-5:** Cytotoxicity (CC₅₀) of select plant extracts

528

529 **Figure legends**530 **Figure 1:** Distribution of plant parts frequently used for edible purposes, compared to the
531 active parts found to have antimicrobial activity in the present study532 **Figure 2:** Antimicrobial activity score with different solvents533 **Figure 3:** Number of edible plants with at least one extract showing activity (>50%) against
534 the tested organisms535 **Figure 4:** Clustering and heat maps of phytochemicals of select extracts on TLC

536

Table-1: List of edible plants of Odisha, and their uses

Plant code No.	Voucher No.	Plant name	Family	Parts edible	Mode of consumption and use	References
P-1	NOU KL088/2014	<i>Aegle marmelos</i> (L.) Corrêa	Rutaceae	Fruits Leaves	Pulp of the ripe fruit is consumed as beverage for body cooling during the summer. Unripe fruits are half-burnt in a fire and consumed as food.	(Mahapatra and Panda, 2012)
P-2	NOU KL091/2014	<i>Ardisia solanacea</i> (Poir.) Roxb.	Primulaceae	Fruits	Juice of ripe fruit is a favorite drink of tribal people.	(Misra and Misra, 2016)
P-3	NOU KL016/2014	<i>Argyreia speciosa</i> (L. f.) Sweet	Convolvulaceae	Young leaves Roots	Young leaves are fried and eaten. Dried roots are taken as powder for long life. Also young leaves are edible.	Current study
P-4	NOU KL095/2014	<i>Argyreia nervosa</i> (Burm. f.) Bojer	Convolvulaceae	Leaves	Leaves are eaten raw as well as fried as a vegetable.	(Misra and Misra, 2013)
P-5	NOU302/2013	<i>Bauhinia racemosa</i> Lam.	Fabaceae	Young leaves	Boiled and fried leaves are taken as curry.	(Panda, Das, Dash, Ghosh, 2015)
P-6	NOU KL092/2014	<i>Bauhinia semla</i> Wunderlin	Leguminosae	Leaves	Young leaves are fried and consumed as vegetables.	(Misra and Misra, 2013)
P-7	NOU KL071/2014	<i>Boerhavia diffusa</i> L.	Nyctaginaceae	Young leaves	Young leaves are fried, then eaten as vegetables.	(Misra and Misra, 2013)
P-8	NOU KL080/2014	<i>Bridelia retusa</i> (L.) A.Juss.	Phyllanthaceae	Fruit	Ripe fruits are eaten raw.	(Mahapatra and Panda, 2012) (Sinha and Lakra, 2005)

P-9	NOU207/2012	<i>Buchanania lanzan</i> Spreng.	Anacardiaceae	Ripe fruit & seed kernel	Ripe fruits edible; seed kernels used as confectionary.	(Mahapatra and Panda, 2012) (Sinha and Lakra, 2005) (Bandana and Debabrata, 2015)
P-10	NOU290/2012	<i>Butea monosperma</i> (Lam.) Taub.	Leguminosae	Young roots	Young roots are used as flour for preparation of bread. Watery sap oozing out from the stem upon cutting is used for drinking.	(Misra and Misra, 2016)
P-11	NOU KL013/2014	<i>Calendula officinalis</i> L.	Compositae	Leaves Flowers	The petals are edible and can be used fresh in salads or dried to colour cheese, or to replace saffron.	Current study
P-12	NOU315/2013	<i>Careya arborea</i> Roxb.	Lecythidaceae	Fruits	Ripe fruits are often cooked.	(Misra and Misra, 2016)
P-13	NOU304/2013	<i>Casearia graveolens</i> Dalze 1	Salicaceae	Leaves and twigs Fruit	Used as cooked vegetable.	(Sinha and Lakra, 2005)
P-14	NOU KL009/2014	<i>Centella asiatica</i> (L.) Urb.	Apiaceae	Leaves	Ripe fruits are edible. Raw leaves are taken on empty stomach as pickles or chutney.	(Panda, 2014) (Misra and Misra, 2013) (Panda, 2014)
P-15	NOU KL018/2014	<i>Cinnamomum zeylanicum</i> Blume	Lauraceae	Bark	Powdered bark is used as spice.	Current study
P-16	NOU KL017/2014	<i>Clitoria ternatea</i> L.	Leguminosae	Leaves	The young pods are edible and used as vegetables.	Current study
P-17	NOU KL001/2014	<i>Curculigo orchiodes</i> Gaertn.	Hypoxidaceae	Rhizome	Rhizomes are edible.	(Panda, 2014)
P-18	NOU KL073/2014	<i>Curcuma amada</i> Roxb.	Zingiberaceae	Rhizome	Used as spice in curry. Also used as pickles in salads.	Current study
P-19	NOU KL096/2014	<i>Dillenia aurea</i> Sm.	Dilleniaceae	Fruits	Raw and ripe fruits are edible.	(Sinha and Lakra, 2005)
P-20	NOU062/2012	<i>Dillenia pentagyna</i> Roxb.	Dilleniaceae	Leaves Fruits	Leaves are eaten by elephants. Ripe fruits are consumed raw.	(Misra and Misra, 2016) (Mahapatra and Panda, 2012) (Bandana and Debabrata,

				Flowers	Flowers are edible.	2015)
P-21	NOU057/2012	<i>Diospyros malabarica</i> (Desr.) Kostel.	Ebenaceae	Fruits	Ripe fruits are sweet and consumed raw.	(Sinha and Lakra, 2005) (Misra and Misra, 2016) (Panda, 2014) (Bandana and Debabrata, 2015) (Mahapatra and Panda, 2012) (Misra and Misra, 2016)
P-22	NOU KL067/2014	<i>Emblica officinalis</i> Gaertn.	Phyllanthaceae	Fruits	Fruits are eaten as berry. Juice is taken for several health remedies including glowing skin.	
P-23	NOU KL052/2014	<i>Enydra fluctuans</i> DC.	Compositae	Leaves	Raw leaves are taken as salad.	Current study
P-24	NOU KL090/2014	<i>Gardenia latifolia</i> Aiton	Rubiaceae	Fruits	Fleshy pulp of the ripe fruits is commonly consumed by tribal peoples.	(Misra and Misra, 2016)
P-25	NOU KL085/2014	<i>Glochidion lanceolarium</i> (Roxb.) Voigt	Phyllanthaceae	Fruits	Fruits are commonly eaten by birds. Powder of fruits is used for stomach ulcer.	Current study
P-26	NOU KL064/2014	<i>Gymnema sylvestre</i> (Retz.) R.Br. ex Sm.	Apocynaceae	Leaves	Leaves are used for controlling obesity with tea.	Current study
P-27	NOU KL066/2014	<i>Ipomoea digitata</i> L.	Convolvulaceae	Roots	Roots are consumed as raw food.	Current study
P-28	NOU310/2013	<i>Lannea coromandelica</i> (Houtt.) Merr.	Anacardiaceae	Fruits	Ripe fruits are eaten raw	(Misra and Misra, 2016)
P-29	NOU KL039/2014	<i>Limonia acidissima</i> Groff	Rutaceae	Fruits	Ripe fruit pulp is eaten and also drunk as juice. Pulp of unripe fruit is made into pickle.	(Misra and Misra, 2016)
P-30	NOU KL058/2014	<i>Linum usitatissimum</i> L.	Linaceae	Seeds	Used as edible oil.	Current study
P-31	NOU KL037/2014	<i>Madhuca longifolia</i> (J.Koenig ex L.) J.F.Macbr.	Sapotaceae	Fruits	Fermented fruits are drunk as wine.	(Bandana and Debabrata, 2015)
				Seeds	Oil obtained from the seeds is used for cooking	(Misra and Misra, 2013)
				Flowers	Flowers are taken as a remedy for constipation	(Mahapatra and Panda, 2012)
P-32	NOU KL022/2014	<i>Mimusops elengi</i> L.	Sapotaceae	Fruits	Ripe fruits are edible.	(Sinha and Lakra, 2005) (Panda, 2014)

P-33	NOU KL061/2014	<i>Ocimum tenuiflorum</i> L.	Lamiaceae	Leaves	Leaves are used as flavouring in salads and desserts. Commonly used with tea.	Current study
P-34	NOU KL072/2014	<i>Piper longum</i> L.	Piperaceae	Fruits	Fruits are powdered and used as vegetable spice. Also, for curing hiccups.	Current study
P-35	NOU KL041/2014	<i>Pithecellobium dulce</i> (Rox b.) Benth.	Leguminosae	Fruits	Fruits are taken as berry. Sweet mature seeds are eaten raw.	(Misra and Misra, 2016)
P-36	NOU KL089/2014	<i>Protium serratum</i> (Wall. ex Colebr.) Engl.	Burseraceae	Fruits	Ripe fruits are acidic and eaten as pickles.	(Mahapatra and Panda, 2012) (Misra and Misra, 2016)
P-37	NOU KL012/2014	<i>Punica granatum</i> L.	Lythraceae	Fruits Seeds	Ripe fruits are often consumed. Seeds are taken after grinding as juice for the treatment of diabetes and diarrhoea.	Current study
P-38	NOU103/2012	<i>Rubus ellipticus</i> Sam.	Rosaceae	Fruits	Ripe fruits are consumed raw.	(Misra and Misra, 2016)
P-39	NOU KL024/2014	<i>Semecarpus anacardium</i> L. f.	Anacardiaceae	Fruits	Hypocarp is eaten when ripe, kernel also edible.	(Mahapatra and Panda, 2012) (Sinha and Lakra, 2005) (Bandana and Debabrata, 2015)
P-40	NOU103/2012	<i>Symplocos cochinchinensis</i> (Lour.) S.	Symplococaceae	Leaves	The leaves have been used as a vegetable salad, and along with cucumber juice for the treatment of diabetes.	(Vedand Goraya, 2007)
P-41	NOU KL076/2014	<i>Syzygium nervosum</i> A.Cun n. ex DC.	Myrtaceae	Leaves & bark Fruits	Both leaves and bark powder are taken as anti-diabetic. Fruits are consumed as raw food.	(Misra and Misra, 2016)
P-42	NOU KL050/2014	<i>Tamarindus indica</i> L.	Leguminosae	Leaves Flower Fruit	Leaves and flowers are cooked as vegetables. Fruits are used as a component of a savory dishes and most frequently	(Misra and Misra, 2013) (Sinha and Lakra, 2005) Current study

P-43	NOU KL003/2014	<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn.	Combretaceae	Bark	as chutney. ¹ Bark powder is taken as medicine for diarrhoea.	(Panda, 2014)
P-44	NOU KL075/2014	<i>Terminalia chebula</i> Retz.	Combretaceae	Fruits	Fruits are taken in powder form for stomach diseases.	(Misra and Misra, 2016) (Mahapatra and Panda, 2012)
P-45	NOU KL057/2014	<i>Tinospora cordifolia</i> (Willd.) Miers	Menispermaceae	Leaves	Leaves are taken as vegetable.	Current study
P-46	NOU KL043/2014	<i>Woodfordia fruticosa</i> (L.) Kurz	Lythraceae	Fruits	Ripe fruits consumed as raw food.	(Misra and Misra, 2016) (Bandana and Debabrata, 2015)

¹ <https://en.wikipedia.org/wiki/Chutney>

Table-2: Antimicrobial activity of 25 plants showing strong inhibition (>80% at a concentration of 1 mg/mL) against Gram-positive food-borne pathogens

Plant name	Part	<i>B. cereus</i>			<i>S. faecalis</i>			<i>L. innocua</i>			<i>M. luteus</i>		
		A	W	E	A	W	E	A	W	E	A	W	E
<i>Aegle marmelos</i>	Leaf	-7	66	5	0	<u>100</u>	4	-7	50	<u>123</u>	<u>100</u>	<u>115</u>	<u>102</u>
<i>Bauhinia racemosa</i>	Leaf	<u>100</u>	-8	<u>105</u>	-7	-8	54	27	-14	<u>82</u>	53	-19	<u>81</u>
<i>Boerhavia diffusa</i>	Leaf	-11	<u>109</u>	-24	0	8	-21	69	50	34	<u>97</u>	50	<u>83</u>
<i>Careya arborea</i>	Leaf	<u>101</u>	64	<u>105</u>	27	21	-3	<u>101</u>	54	<u>127</u>	<u>100</u>	73	<u>138</u>
<i>Casearia graveolens</i>	Leaf	<u>115</u>	72	<u>96</u>	<u>90</u>	32	66	<u>100</u>	77	<u>96</u>	<u>98</u>	<u>94</u>	<u>110</u>
<i>Curculigo orchoides</i>	Rhizome	-19	-15	-19	10	44	21	<u>95</u>	-4	52	<u>96</u>	-7	75
<i>Curcuma amada</i>	Rhizome	-	0	-22	21	-33	-4	50	19	<u>92</u>	-26	-25	<u>101</u>
<i>Diospyros malbarica</i>	Leaf	-	-	<u>94</u>	-	-	<u>92</u>	-	-	66	-	-	<u>104</u>
<i>Embllica officinalis</i>	Fruits	2	20	-14	32	44	33	<u>87</u>	68	<u>98</u>	34	29	54
<i>Gardenia latifolia</i>	Leaf	39	25	23	40	76	12	<u>106</u>	73	73	76	24	<u>92</u>
<i>Gymnema sylvestre</i>	Leaf	-4	0	-20	0	12	48	<u>81</u>	-3	63	<u>99</u>	-18	<u>98</u>
<i>Limonia acidissima</i>	Leaf	5	2	-18	-18	14	30	<u>87</u>	16	<u>81</u>	<u>92</u>	6	<u>104</u>
<i>Madhuca longifolia</i>	Leaf	-1	53	-21	-23	<u>83</u>	37	-26	<u>82</u>	52	49	-8	<u>81</u>
<i>Meyera fluctuans</i>	Leaf	-1	-12	-15	-12	7	0	<u>98</u>	30	20	<u>99</u>	0	0
<i>Mimusops elengi</i>	Leaf	-2	45	31	-16	54	56	32	<u>101</u>	<u>100</u>	<u>81</u>	43	<u>110</u>
<i>Ocimum sanctum</i>	Seeds	-11	<u>90</u>	0	0	-18	24	12	<u>100</u>	71	70	50	71
<i>Protium serratum</i>	Leaf	-4	0	11	25	11	10	50	-34	<u>91</u>	<u>132</u>	-10	<u>101</u>
<i>Punica granatum</i>	Seeds	-1	-8	-21	-19	-22	46	74	11	<u>91</u>	27	-27	<u>91</u>
<i>Rubus ellipticus</i>	Leaf	1	<u>102</u>	<u>95</u>	7	69	58	19	<u>91</u>	57	<u>101</u>	<u>116</u>	76
<i>Semecarpus anacardium</i>	Leaf	14	6	17	50	<u>106</u>	-7	50	<u>111</u>	-1	<u>109</u>	26	-3
<i>Symplocos cochinchinensis</i>	Leaf	<u>101</u>	31	67	58	-15	19	<u>117</u>	10	58	<u>117</u>	35	78
<i>Tamarindus indica</i>	Leaf	-6	-14	24	-19	2	53	<u>97</u>	21	<u>130</u>	35	-12	74
<i>Terminalia arjuna</i>	Bark	16	51	-22	24	70	39	60	<u>100</u>	-18	68	<u>100</u>	-16
<i>Tinospora cordifolia</i>	Stem	4	44	-7	5	<u>117</u>	20	1	<u>127</u>	<u>84</u>	-5	62	1
<i>Woodfordia fruticosa</i>	Root	29	22	16	-16	62	-9	<u>107</u>	<u>133</u>	66	<u>93</u>	<u>102</u>	50

(-) Not tested; data presented here for only those plants which shows above 80% inhibition (underlined) against at least two pathogens; inhibition of growth in % compared to the solvent, OD at 620 nm; A: acetone, W: water, E: ethanol; results of hexane extracts were excluded due to low activity (for details refer to supplementary material 2)

Table-3: Antimicrobial activity of 25 plants showing strong inhibition (>50% at a concentration of 1 mg/mL) against Gram-negative food-borne pathogens

Plant name	Part	<i>E. coli</i>			<i>P. aeruginosa</i>			<i>S. enterica</i>			<i>S. sonnei</i>		
		A	W	E	A	W	E	A	W	E	A	W	E
<i>Aegle marmelos</i>	Leaf	-2	<u>82</u>	5	-4	-7	-16	0	<u>93</u>	-8	0	12	-5
<i>Ardisia solanacea</i>	Leaf	17	15	-23	-12	<u>149</u>	-11	7	-8	-11	-22	<u>120</u>	-16
<i>Argyreia speciosa</i>	Root	<u>94</u>	<u>64</u>	<u>120</u>	17	<u>50</u>	38	9	0	11	<u>82</u>	<u>62</u>	<u>56</u>
<i>Bauhinia semla</i>	Leaf	29	<u>81</u>	-8	41	-13	42	30	-10	21	-2	<u>73</u>	-9
<i>Boerhavia diffusa</i>	Leaf	-10	<u>107</u>	-15	-8	20	-19	-18	4	-12	<u>59</u>	35	-13
<i>Careya arborea</i>	Leaf	<u>98</u>	<u>53</u>	<u>100</u>	-24	<u>100</u>	15	-2	41	-5	0	<u>79</u>	0
<i>Casearia graveolens</i>	Leaf	-3	40	3	33	<u>97</u>	9	-10	<u>102</u>	-8	-4	-9	-9
<i>Curcuma amada</i>	Rhizome	<u>50</u>	-4	<u>50</u>	-7	17	27	-5	7	0	-16	12	-2
<i>Dillenia aurea</i>	Leaf	38	-2	<u>68</u>	<u>100</u>	<u>110</u>	-4	-11	31	23	-50	18	-24
<i>Dillenia pentagyna</i>	Leaf	-	-	44	-	-	<u>52</u>	-	-	<u>56</u>	-	-	<u>85</u>
<i>Diospyros malbarica</i>	Leaf	-	-	<u>64</u>	-	-	<u>72</u>	-	-	36	-	-	<u>54</u>
<i>Emblica officinalis</i>	Fruits	-3	<u>57</u>	-3	-8	15	-12	-29	15	-11	8	<u>54</u>	29
<i>Gardenia latifolia</i>	Leaf	23	22	<u>84</u>	<u>71</u>	<u>58</u>	<u>81</u>	16	30	10	49	-1	38
<i>Glochidion lanceolarium</i>	Leaf	45	9	<u>81</u>	0	-7	7	20	<u>52</u>	-17	-4	12	-4
<i>Mimusops elengi</i>	Leaf	16	16	1	13	<u>59</u>	<u>76</u>	4	26	15	10	25	<u>54</u>
<i>Ocimum sanctum</i>	Seeds	-17	<u>86</u>	31	-6	<u>50</u>	42	-16	-11	-4	18	-4	-18
<i>Piper longum</i>	Fruits	35	12	<u>59</u>	<u>50</u>	0	-14	47	17	-21	-21	14	-3
<i>Rubus ellipticus</i>	Leaf	1	<u>94</u>	<u>99</u>	-4	-9	-2	-3	-2	-6	-5	34	-6
<i>Semecarpus anacardium</i>	Leaf	<u>61</u>	<u>54</u>	20	15	<u>116</u>	-5	13	<u>80</u>	-4	-13	30	-11
<i>Symplocos cochinchinensis</i>	Leaf	10	19	25	-1	15	-4	-2	-7	-5	36	0	9
<i>Syzygium nervosum</i>	Leaf	<u>50</u>	12	-3	-8	-11	<u>50</u>	14	23	3	-33	-17	-7
<i>Terminalia arjuna</i>	Bark	10	38	<u>72</u>	8	<u>50</u>	-12	28	15	-4	-3	-26	-25
<i>Terminalia chebula</i>	Leaf	-1	29	<u>57</u>	34	<u>6</u>	<u>101</u>	6	17	5	-6	0	1
<i>Tinospora cordifolia</i>	Stem	-7	<u>81</u>	-10	21	<u>50</u>	-14	4	<u>84</u>	-18	-3	2	9
<i>Woodfordia fruticosa</i>	Root	<u>63</u>	48	<u>81</u>	17	31	-11	34	10	-19	<u>53</u>	-23	-7

(-) Not tested; data presented here only for those plants which show above 50% inhibition (underlined) against at least two pathogens; inhibition of growth in % compared to the solvent, OD at 620 nm; A: acetone, W: water, E: ethanol; results of hexane extracts excluded due to limited activity (for details refer to supplementary material 3)

Table 4: MIC₅₀ of plant extracts against select food-borne pathogens

Name of the plant	MIC ₅₀ in µg/mL of extract in various solvents		
	Acetone	Water	Ethanol
<i>Aegle marmelos</i>	439-MI	551-Ec; 327-MI	297-Li; 458-MI
<i>Argyrea speciosa</i>	–	623-Ec; 541-Ss; 300-Bc	63-Ec; 668-Bc
<i>Bauhinia racemosa</i>	377-Bc	480-Ss	459-Bc; 630 -Li; 781-MI
<i>Bauhinia semla</i>	170-Li	600-Ec; 422-Ss	–
<i>Boerhavia diffusa</i>	–	590-Ec; 185-Bc	–
<i>Careya arborea</i>	625-Ec; 332-Bc; 277-Li; 400-MI	777-Pa; 729-Ss;	485-Ec; 258-Bc; 328-Li; 273-MI
<i>Casearia graveolens</i>	259-Bc; 356-Ss; 295-Li; 465-MI	340-Pa; 367-Se	441-Bc; 278-Li; 387-MI
<i>Curculigo orchoides</i>	156-Li; 73-MI	–	–
<i>Curcuma amada</i>	–	–	243-Li; 464-MI
<i>Dillenia pentagyna</i>	–	–	970-Ss; 661-MI
<i>Dillenia aurea</i>	450-Pa	560-Pa	–
<i>Diospyros malbarica</i>	–	–	483-Bc; 309-Sf; 264-MI
<i>Embllica officinalis</i>	247-Li	–	201-Li
<i>Gardenia latifolia</i>	200-Pa; 127-Li; 380-Ec	–	98-Pa; 384-MI
<i>Glochidion lanceolarium</i>	–	2981-Sf	627-Ec
<i>Gymnema sylvestre</i>	576-Li; 276-MI	–	450-MI
<i>Limonia acidissima</i>	197-Li; 385-MI	–	292-Li; 248-MI
<i>Madhuca longifolia</i>	–	619-Ps; 276-Sf; 257-Li	–
<i>Mimusops elengi</i>	244-MI	118-Li	209-Ps; 828-Sf; 208-Li; 370-MI
<i>Meyera fluctuans</i>	474-Li; 417-MI	–	–
<i>Ocimum sanctum</i>	–	619-Ec; 224-Bc; 50-Li	–
<i>Piper longum</i>	520-MI	–	–
<i>Protium serratum</i>	189-MI	–	506-Li; 472-MI
<i>Punica granatum</i>	–	–	540-Li; 493-MI
<i>Rubus ellipticus</i>	282-MI	559-Ec; 559-Bc; 560-Li; 572-MI	527-Ec; 273-Bc; 664-MI
<i>Semecarpus anacardium</i>	593-Ec; 218-MI	916-Ec; 432-Ps; 265-Se; 102-Sf; 65-Li	–
<i>Symplocos cochinchinensis</i>	404-Bc; 267-Li; 450-MI	–	–
<i>Syzygium nervosum</i>	93-Li	–	–
<i>Tamarindus indica</i>	386-Bc	–	–
<i>Terminalia arjuna</i>	–	389-Li; 78-MI	127-Ec
<i>Terminalia chebula</i>	227-MI	185-MI	385-MI
<i>Tinospora cordifolia</i>	–	645-Ec; 562-Se; 547-Sf; 517-Li	630-Li
<i>Woodfordia fruticosa</i>	129-Li; 40-MI	47-Li; 56-MI	541-Ec

Bc: *Bacillus cereus*, Sf: *Streptococcus faecalis*, Li: *Listeria innocua*, MI: *Micrococcus luteus*, Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*, Se: *Salmonella enterica*, Ss: *Shigella sonnei*; for more MIC₅₀ results refer to supplementary material 4

Table-5: Cytotoxicity (CC₅₀) of select plant extracts

Name of the plant	Part tested	RD Cell line (CC ₅₀)	Vero Cell line (CC ₅₀)
<i>Aegle marmelos</i>	Leaf	15.3-A	63.1-A; >100-E
<i>Bauhinia racemosa</i>	Leaf	–	>100-E
<i>Bauhinia semla</i>	Leaf	48.9-A; >100-W; >100-E	–
<i>Boerhavia diffusa</i>	Leaf	76.5-A; 63.1-E	>100-A
<i>Bridelia retusa</i>	Leaf	>100-W	>100-A; >100-E
<i>Buchanania lanzan</i>	Leaf	–	>100-E
<i>Butea monosperma</i>	Flowers	–	>100-E
<i>Careya arborea</i>	Leaf	–	>100-E
<i>Casearia graveolens</i>	Leaf	–	>100-W
<i>Centella asiatica</i>	Leaf	>100-W	–
<i>Clitoria terentea</i>	Leaf	–	>100-A; >100-E
<i>Dillenia pentagyna</i>	Leaf	63-W	20-A; >100-E
<i>Diospyros malbarica</i>	Leaf	–	>100-E
<i>Emblica officinalis</i>	Fruits	50.2-A; 54.7-E	–
<i>Enydra fluctuans</i>	Leaf	76.5-E	–
<i>Gardenia latifolia</i>	Leaf	48.9-A; 20-E	76.5-A; >100-E
<i>Glochidion lanceolarium</i>	Leaf	63.1-A; 15.3-W; >100-E	–
<i>Gymnema sylvestre</i>	Leaf	8.94-E	>100-E
<i>Limonia acidissima</i>	Leaf	>100-W; 10.9-E	40.9-A; 40.9-E
<i>Madhuca longifolia</i>	Leaf	44.7-A; >100-W	>100-A; >100-E
<i>Mimusops elengi</i>	Leaf	52.2-A; 63.1-W	63.1-W; 76.5-E
<i>Ocimum sanctum</i>	Seeds	–	>100-E
<i>Piper longum</i>	Fruits	9.78-A; 9.78-E	4-A
<i>Protium serratum</i>	Leaf	44.7-A; 1.79-E	54.7-A; >100-E
<i>Rubus ellipticus</i>	Leaf	–	59-W; 29-E
<i>Semecarpus anacardium</i>	Leaf	54.7-A; 15.3-W; 54.7-E	–
<i>Symplocos cochinchinensis</i>	Leaf	–	>100-E
<i>Syzygium nervosum</i>	Leaf	36.6-A; 20-W; 20-E	>100-A; 8.9-E
<i>Tamarindus indica</i>	Leaf	–	>100-E
<i>Terminalia arjuna</i>	Bark	–	>100-W
<i>Terminalia chebula</i>	Leaf	76.5-A; 20-W; 15.3-E	–
<i>Tinospora cordifolia</i>	Stem	48.9-A	63.1-A; >100-E
<i>Woodfordia fruticosa</i>	Root	>100-A; 76.5-W; 48.9-E	–

A: acetone, E: ethanol, W: water

Title- Antimicrobial activity of select edible plants against food-borne pathogens

- The antimicrobial activity was studied of 46 plants against 8 food-borne pathogens.
- Ten plants showed broad-spectrum -, while 28 exhibited narrow-spectrum activities.
- Fourteen plant extracts showed cytotoxicity against both RD and Vero cell lines.
- Ten edible plants have potential as herbal preservatives in the food industry.