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Research Article

Comparative Sensitivity of *Salmonella* Isolates from Clinical Infections in Animals and Birds to Herbal and Conventional Antimicrobials -

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ABSTRACT

Introduction: Salmonellosis is an important zoonosis. However, little is known about comparative sensitivity of *Salmonella* to conventional and herbal antimicrobial drugs.

Methods: Sensitivity assays for 16 herbal and 25 conventional antimicrobials on 101 isolates of 21 serovars of *Salmonella enterica* ssp. *enterica* and one strain of *S. enterica* ssp. *indica* and two of *S. enterica* ssp. *salamae* from clinical cases (46) and repository (55) strains, were done using disc diffusion assay and interpreted as per CLSI guideline.

Results: The sensitivity data revealed that 57.4% of *Salmonella* isolates had Multiple-Drug-Resistance (MDR), 24.6% produced Extended-Spectrum-B-Lactamases (ESBL) and strain each was resistant to carbapenems and moxalactam. There was no significant difference among repository and clinical strains with respect to ESBL and MDR traits. Non-MDR salmonellae were more often (p = 0.04) resistant to Thyme Oil (TO) but less often (p = 0.03) to Rosewood Oil (RWO) than strains resistant to 4 or 5 antibiotics. Salmonellae resistant to 10-11 herbal antimicrobials were more often (p = 0.02) sensitive to cotrimoxazole than those resistant to 8 herbal antimicrobials. Ceftazidime and aztreonam resistance was more common ($p \leq 0.03$) among salmonellae resistant to ≤7 herbal antimicrobials than salmonellae resistant to >7 herbal antimicrobials. Repository samonellae were more ($p < 0.05$) often resistant than those from clinico-pathological samples to Holy Basil Oil (HBO), *Zanthoxylum Rhetsa* Essential Oil (ZEO), cotrimoxazole, ceftazidime and colistin. Cinamaldehyde, carvacrol, Ajowan Oil (AO), Cinnamon Oil (CO), TO, HBO, Agar Wood Oil (AWO), Patchouli Essential Oil (PEO), Marjoram Essential Oil (MEO), Sandal Wood Oil (SWO), Guggul Oil (GO) and ZEO inhibited 95, 94, 93, 91, 89, 87, 1, 2, 5, 5, 8 and 9strains, respectively. Among antibiotics, azithromycin was the least effective followed by nalidixic acid, amoxicillin, ampicillin and amoxicillin + clavulanic acid inhibiting 16, 68, 76, 80 and 81 of the *Salmonella* strains tested, respectively. Cefepime, moxalactam, imipenem and meropenem inhibited growth of 100 strains and ceftriaxone, cefotaxime + clavulanic acid, aztreonam, tiecycline, cefoxiti, chloramphenicol, piperacillin + tazobactam and gentamicin failed to inhibit 2, 3, 3, 3, 4, 5, 6 and 7 strains, respectively.

Conclusion: The study revealed that on animal and poultry salmonellae tetracycline, one of the most commonly used antibiotic in veterinary practice, may be useless. However, cephalosporins and quinolones still hold ground for their use in therapeutics. Among herbal antimicrobials, herbals containing carvacrol (AO and TO), Cinnamaldehyde (CO) and to some extent Eugenol (HBO) may be explored for development of useful therapeutic preparations to curtail growth of salmonellae.

Keywords: *Salmonella enterica* ssp. Enterica*;* *S. enterica* ssp. Salamae; *S. enterica* ssp. Indica; Cinnamon oil; Holy basil oil; Ajowan oil; MDR; MHAR; MRI; HMRI

ABBREVIATIONS

S: *Salmonella enterica* ssp. *enterica*; AMR: Antimicrobial Drug Resistance; MDR: Multiple Drug Resistance; MHAR: Multiple Herbal Drug Resistance; MRI: Multiple Drug Resistance Index; HMRI: Multiple Herbal Drug Resistance Index

INTRODUCTION

Salmonella has long been recognized as an economically important zoonotic pathogen all over the world. Interest in *Salmonella* has heightened in recent years due to the devastating impact of salmonellosis on poultry industry and the globalization of trade [1]. It is a menace to the food industry and havoc to animal and human health. Even stringent control measures have proven futile against it [2]. Despite the immense amount of research on immunoprophylaxis against salmonellosis, much less could be achieved. Till date, no single vaccine is available which can protect against majority of the ever increasing number of *Salmonella* serovars [3]. Furthermore, it is difficult to say where and which serovar will dominate tomorrow as *S. enterica* ssp. *enterica* ser Typhimurium (*S.* Typhimurium) was prevalent some time back in India [4] and *S.* Enteritidis in Europe and America [5], but later *S.* Weltevreden emerged as zoonotic serovar globally [6] and *S.* Indiana in China replaced *S.* Typhimurium, *S.* Derby and *S.* Agona [7,8].

Salmonellosis, one of the most common diseases in humans, dairy cattle, beef cattle, and poultry, is not uncommon in sheep, pigs, goats, rabbits, dogs, seabirds, rodents, porpoises, cats, horses, and aquatic animals [3]. All *Salmonella* are primarily transmitted through the fecal-oral route. Other modes of zoonotic transmission include direct contact with livestock, wildlife, or pets, especially cats and turtles. Animal-animal transmission happens at the farm through contaminated food and water sources, pastureland, or contact with newly acquired animals [2,9,10]. Salmonellosis in animals is usually reported in four forms;

Enteritis

Foul smelling watery faeces, with or without fibrin, mucous and blood. Death is due to dehydration, electrolyte loss and imbalance of acid-base.

Septicemia

Fever, inappetence and pneumonia, mostly in infants and young leading to loss of production. Localization may lead to meningitis or poly-arthritis. In sub-acute form, disease may result into slow resolution.

Abortions

In pregnant animals mostly due to host adapted serovars. In early pregnancy resorption of fetus without abortion or apparent illness may also occur,

Localised infections

Rarely reported, abscess, wound infection, and urinary tract infection. In poultry birds, salonellosis may be caused by several serovars but Fowl Typhoid (FT) caused by *S.* Gallinarum mainly affects adult birds. It is characterized by high mortality in early stages of outbreaks while Pullorum Disease (PD) caused by *S. Pullorum*, is vertically transmitted disease affecting primarily chicks in first few days of life characterized by white bacillary diarrhea, besides it also cause high dead-in-shell chicks [2]. India is a hyper-endemic state for typhoid and paratyphoid [1] but little is understood about antimicrobial resistance of salmonellae causing infections in animals in India.

Emergence and global spread of Multiple-Drug-Resistant (MDR) and extensively drug-resistant *Salmonella* serovars ([7,8,11,12] and causing huge economic loss lead us to search for any alternative for antibiotics and herbal antimicrobials are one which are known for their vast potential [13,14]. In recent years, herbs or their active ingredients are reported as potential alternatives of antibiotics [13,14]. Use of herbs and herbal compounds to control bacterial diseases including salmonellosis has been documented in various communities using traditional therapies [15]. Herbs has not only used in human medicine as antimicrobials but in veterinary medicine as well [15]. A range of herbs has been shown effective against *Salmonella* isolates from food too [6]. Therefore, this study was undertaken to assess the herbal antimicrobials for their potential as anti-*Salmonella* agents. In this study, *Salmonella* isolates in repository of National *Salmonella* Centre (Vet.), ICAR-IVRI, Izatnagar, India and *Salmonella* recently isolated from clinico-pathological sample received in Clinical epidemiology laboratory of ICAR-IVRI, Izatnagar were tested for their sensitivity to sixteen herbal and 25 conventional antimicrobial drugs.

MATERIALS AND METHODS

Isolation and identification of *Salmonella*

Samples received from clinical pathology of the institute (swabs, heart blood, tissue samples, and stomach contents of aborted fetuses etc.) were processed for isolation and identification of the pathogens in clinical epidemiology laboratory of the institute using standard protocol [17]. All *Salmonella* isolates were sent to National *Salmonella* Centre (Vet.), Izatnagar for serotyping. Forty six serotyped and confirmed isolates of *Salmonella* from various pathological conditions in different animals and birds and 55 strains of different serovars from repository of National *Salmonella* Centre (Vet.) were included in the study (Table 1) to evaluate their sensitivity to commonly used antibiotics and herbal antimicrobials. Before starting the sensitivity assays, all revived isolates were again tested for their growth and biochemical characteristics as per standard procedures [18] using criteria detailed in *Bergey's Manual of Determinative Bacteriology* [3]. All the cultures were maintained in the laboratory on nutrient agar slants till the end of the study.

Antimicrobial sensitivity assay

Antimicrobial sensitivity of all 101 *Salmonella* isolates (strains) was tested with disc diffusion assay and interpreted as sensitive or resistant as per CLSI guideline [19] against antimicrobial discs (Difco BBL, USA) for amoxicillin (30µg), amoxicillin (30µg) + clavulanic acid (10µg), ampicillin (10µg), azithromycin (15µg), aztreonam (30µg), cefepime (30µg), cefotaxime (10µg), cefotaxime (10µg) + clavulanic acid (10µg), cefoxitin (10µg), ceftazidime (30µg), ceftriaxone (10µg), chloramphenicol (25µg), colistin (10µg), cotrimoxazole (25µg), doxycycline (30µg), enrofloxacin (10µg), erythromycin (15µg), gentamicin (30µg), imipenem (10µg), meropenem (10µg), moxalactam (15µg), nitrofurantoin (300µg), piperacillin (100µg), piperacillin (100µg) + tazobactam (10µg), tetracycline (30µg) and tigecycline (15µg) on Mueler Hinton agar (MHA, Difco) plates. An *E. coli* strain (E-382) was used as control reference antibiotic sensitive strain. Salmonellae resistant to three or more classes of therapeutically used antimicrobials were designated Multi-Drug-Resistant (MDR). Extended Spectrum Β-Lactamase (ESBL) production ability of all the salmonellae was determined using ESBL E-test strips (Biomerieux, France) as per direction of the supplier. The Multiple Antibiotic Resistance (MAR) indices of test *Salmonella* were calculated as; number of drugs resisted divided by number of drugs tested.

All salmonellae were also tested for their sensitivity to discs (1 µL of test substance in each disc) of Agar Wood (Aquilaria sinensis) Oil (AWO), Ajowan (Trachyspermum ammi) Oil (AO), Betel (Piper betle) Leaf Oil (BLO), carvacrol (Sigma, USA), cinnamaldehyde (Sigma, USA), Cinnamon (Cinnamomum verum) Oil (CO), citral (Sigma, USA), Guggul (Commiphora mukul) Oil (GO), Holy Basil (Ocimum sanctum) Oil (HBO), Lemon Grass (Cymbopogon citratus) Oil (LGO), Marjoram (Origanum majorana) Essential Oil (MEO), Patchouli (Pogostemon cablin) Essential Oil (PEO), Rose Wood (Dalbergia latifolia) Oil (RWO), Sandal Wood (Santalum album) Oil (SWO), Thyme (Thymus vulgaris) Oil (TO) and Zanthoxylum rhetsa Essential Oil (ZEO). All herbal oils except guggul oil with >99.5% purity were received from Shubh Flavours and Fragrance Ltd, New Delhi while pure guggul oil was received as a kind gift from Dr. MZ Siddiqui, Processing and Product Development Division, ICAR - Indian Institute of Natural Resins & Gums, Namkum, Ranchi, India. The discs loaded with 1µL of herbal compound/ oil were prepared as described earlier and stored in sealed vials at 4°C till used for disc diffusion assays [20]. Any zone of growth inhibition around herbal disc was measured in mm and the isolate was classified as sensitive, if no visible growth inhibition zone (ZI) was there the isolated was considered as resistant. Similar to MAR and MRI, herbal MAR (MHAR) and herbal MRI (HMRI) were calculated for all salmonellae tested. In evaluating antimicrobial activity of different herbal compounds a fixed amount of all the herbals (1 µL/ disc) was used so that a comprehensive view of their comparative activity can be assessed as suggested in earlier publications [13].

STATISTICAL ANALYSIS

Salmonella isolates (strains) sensitivity data was analysed in Microsoft Office Excel worksheet for correlation among ZI diameters measured in mm against different antimicrobials, odds ratio and Chi-square test for different variable as source of isolation, association with different ailments and serovars of salmonellae.

RESULTS

Sensitivity assays of 101 *Salmonella* of 21 serovars of *S. enterica* ssp. *enterica* (*S.*) and one strain of *S. indica* and two of *S. salamae* revealed that 58 (57.4%) isolates had MDR, 25 (24.6%) produced ESBL and one each was resistant to carbapenems (*S. salmae*) and moxalactam (*S.* Typhimurium). A total of nine reference strains, two *S.* Abortusequi and one each of *S. Paratyphi* A, *S. Paratyphi* B, *S. Typhi*, *S. Pullorum*, *S. Typhimurium*, *S. Kentucky* and *S. Illinois* serovars produced ESBL. A total of 16 isolates of clinical origin (five *S.* Typhimurium from poultry, eight from domesticated animals including 2 *S.* Adelaide, 2 *S.* Kentucky and 2 *S.* Typhimurium, 1 *S.* Abortusequi and 1 *S. salamae*) and three from wild animals including *S. I.* 6,8:-, *S. indica*, *S. salamae*) produced ESBL. Only one isolate of *S. salamae* isolated from lung aspirate of a Himalayan bear died of pneumonia was resistant to meropenem and imipenem (MIC 16µg/ mL) along with 13 more antibiotics (MRI, 0.6) and 9 herbal antimicrobials.

Non-MDR salmonellae were more often ($p = 0.04$) resistant to TO but significantly ($p = 0.03$) less often to RWO than strains resistant to 4 to 5 antibiotics. Ceftazidime and aztreonam resistance was more often ($p \leq 0.03$) detected in *Salmonella* resistant to ≤7 herbal antimicrobials than salmonellae resistant to >7 herbal antimicrobials; however, picture was in total inverse with respect to sensitivity of salmonellae isolates to amoxicillin and amoxicillin+ clavulanic acid.

Repository samonellae were more often resistant ($p < 0.05$) than those from clinico-pathological samples to HBO, MEO, cotrimoxazole, ceftazidime and colistin while order was reverse with respect to their resistance towards ampicillin, aztreonam, enrofloxacin, azithromycin, cefotaxime, cefoxitin and piperacillin.

None of the antibiotics and herbal antimicrobial was able to inhibit all salmonellae in the study (Table 2,3). Cinamaldehyde inhibiting 95 strains was followed by carvacrol, AO, CO, TO and HBO inhibiting 94, 93, 91, 89 and 87 strains, respectively while AWO, PEO, MEO, SWO, GO and ZEO could inhibit only 1, 2, 5, 5, 8 and 9 strains of *Salmonella*, respectively (Table 2). Among antibiotiotics azithromycin was the least effective on *Salmonella* followed by nalidixic acid, amoxicillin, ampicillin and amoxicillin + clavulanic acid inhibiting 16, 68, 76, 80 and 81 strains, respectively (Table 3). Cefepime, moxalactam, imipenem and meropenem inhibited growth of 100 strains while ceftriaxone, cefotaxime + clavulanic acid, aztreonam, tiecycline, cefoxiti, chloramphenicol, piperacillin + tazobactam and gentamicin failed to inhibit 2, 3, 3, 3, 4, 5, 6 and 7 strains, respectively (Table 3).

All the isolates from domesticated and wild animals were sensitive to AO, HBO, CNH, carvacrol, TO and CO but resistant to GO (Table 2). *Salmonella* isolates from poultry birds were more often ($p < 0.05$) sensitive to nitrofurantoin, BLO and GO while less often sensitive to amoxicillin + clavuanic acid, AO, CO, TO, MEO and carvacrol than isolated from diseased domestic animals (Table 2).

Salmonellae from domestic animals were often ($p < 0.05$) more sensitive to ampicillin, amoxicillin, amoxicillin + clavulanic acid, ceftazidime, enrofloxacin, nitrofurantoin and tigecycline than those isolated from wild animals. However, *Salmonella* from wild animals were more often ($p < 0.05$) sensitive to BLO and RWO than those from domestic animals but more often ($p < 0.05$) resistant to ampicillin, aztreonam, cefepime, ceftazidime, enrofloxacin, imipenem, meropenem, piperacillin and tigecycline than salmonellae isolated from samples of diseased poultry birds (Table 3).

Among *Salmonella* isolates from horses were less often ($p < 0.05$) sensitive to aztreonam, cefotaxime, piperacilin and piperacillin + tazobactam than those from pigs, and for aztreonam, nitrofurantoin and piperacillin than those from poultry birds. However, salmonellae from horses were more often sensitive than those from poultry birds to MEO ($p < 0.05$).

Most of the salmonellae irrespective of association with ailments were resistant to citral, GO, RWO, MEO, ZEO, AO, PEO and azithromycin (Table 4). Resistance to AO, cinnamaldehyde, carvacrol, meropenem, imipenem and moxalactam was detected only in salmonellae isolated from death cases (Table 4). All 55 repository strains were sensitive to meropenem, imipenem, cefotaxime, cefotaxime + clavulanic acid, ceftriaxone, cefepime and aztreonam (Table 5). Except for a few, there was no significant difference ($p > 0.05$) in sensitivity of clinical and repository *Salmonella* isolates to herbal and conventional antimicrobials (Table 4,5). Resistance to HBO and tigecycline was detected only in a few isolates, and all resistant isolates were from diarrhoeic cases. *Salmonellae* from cases of death were significantly ($p = 0.01$) more often resistant to ZEO and produced ESBL ($p = 0.03$) than those from diarrhoea and abortion cases. Nalidixic acid and cefoxitin resistance was more common ($p = 0.02$) in salmonellae isolated from death cases than those from diarrhoeic cases. *Salmonella* isolates from death cases were significantly ($p = 0.03$) more often resistant to amoxicillin + clavulanic acid than those from other ailments but isolates from abortion cases were significantly more often resistant to piperacillin + tazobactam ($p = 0.01$) than those from death and diarrhoeic cases.

There was insignificant negative correlation ($r = -0.04$; $p > 0.1$) in MAR and MHAR, and MRI and MHRI of salmonellae. However, many of the herbal and conventional antimicrobials had significant positive or negative correlation among their ZIs produced against *Salmonella* (Table 6). Agar wood oil ZIs were negatively correlated ($p = 0.05$) with ZIs of BLO and HBO, ZIs of PEO with ZIs of HBO, and ZIs of GO with ZIs of carvacrol (Table 6). The ZIs induced by azithromycin ceftazidime and enrofloxacin had significant ($p \leq 0.05$) negative correlation with ZIs induced by BLO and carvacrol.

Resistance pattern was not similar for salmonellae of different serovars. *Salmonella* Abortusequi strains were more commonly resistant ($p \leq 0.05$) to BLO than *S.* Gallinarum, *S.* Paratyphi A, *S.* Pullorum, *S.* Tyhi and *S.* Typhimurium, to CO than *S.* Kentuky, and to PEO and SWO than *S.* Paratyphi A, to GO and nalidixic acid than *S.* Typhimurium; and to azithromycin than *S.* Virchow strains. *Salmonella* Abortusequi strains were more often ($p \leq 0.05$) sensitive to tetracycline than *S.* Kentucky and S. Virchow strains, to enroflxacin than *S.* Kentucky and *S.* Paratyphi A, and to citral, colistin, cotrimoxazole, nalidixic acid and tigecycline than *S.* Virhow isolates.

Salmonella Gallinarum isolates were more commonly ($p \leq 0.05$) resistant to AO, CO, TO and carvacrol but more often ($p \leq 0.05$)

Table 1: Salmonellae tested in the study from different sources.

Source/ associated ailment	*Salmonella enterica* ssp. *enterica* (*S.*) serovars or *Salmonella enterica* subspecies strains isolated from different sources
Abortion (9)	Abortusequi 5 from aborted foals, Dublin 3 from aborted buffalo calves, Typhimurium 1 from aborted foal, all isolation were from stomach contents of aborted foeti.
Abscess (1)	*S. enterica* ssp. *salamae*, in pus swab of a horse
Death (21)	Adelaide 2, spleen of pigs; Gallinarum 4, heart blood of poultry; S. enterica ssp. *indica*, intestinal contents of tortoise; Kentucky 3, 2 from spleen of pigs and one from intestinal contents of a tiger; *S. enterica* ssp. *salamae* 1, lung aspirate of Himalayan bear died of pneumonia; Typhimurium 10, 8 from heart blood of poultry birds, one from heart blood of pig, one from gall bladder of cattle.
Diarrhoea (13)	6,8:- one from tiger faecal swab; 6,7:- one from faecal swab of poultry bird; Kentucky 7, from faecal swabs of piglets; Miyazaki one from faecal swab of poultry bird; Typhimurium 3, two from poultry faecal swabs and one from piglet faecal swab
Nasal Catarrh (1)	*S. enterica* ssp. *indica* from nasal swab of a pig
Post surgery wound (1)	Typhimurium from pus swab of a buffalo
Repository (NSC Vet.) strains (55), isolated before 2005	Abortusequi 8, Anatum 1, Choleraesuis 2, Deversoir 1, Enteritidis 1, Gallinarum 1, Illinois 1, Infantis 1, Javiana 1, Kentucky 2, Paratyphi A 7, Paratyphi B 2, Pullorum 6, Typhi 5, Typhimurium 9 and Virchow 7.

NSC Vet.: National *Salmonella* Centre (Veterinary)

Table 2: Herbal antimicrobial resistance pattern and drug resistance indices of *Salmonella enterica* isolated from different sources.

Antimicrobials and Resistance Measures	**Percent Resistant Strains from Different Sources**					
	Reference (55)	*Salmonella* from Clinical samples from				Total (101)
		Poultry birds (16)	Domesticated animals (26)	Wild animals (4)	Total (46)	
Agar-wood oil	98.18	100.00	100.00	100.00	100.00	99.01
Ajowan oil	7.27	25.00	0.00	0.00	8.70	7.92
Betel leaf oil	67.27	56.25	84.62	25.00	69.57	68.32
Carvacrol	7.27	18.75	0.00	0.00	6.52	6.93
Cinnamaldehyde	9.09	6.25	0.00	0.00	2.17	5.94
Cinnamon oil	12.73	18.75	0.00	0.00	6.52	9.90
Citral	61.82	62.50	76.92	50.00	69.57	65.35
Guggul oil	90.91	81.25	100.00	100.00	93.48	92.08
Holy basil oil	21.82	12.50	0.00	0.00	4.35	13.86
Lemongrass oil	83.64	81.25	69.23	50.00	71.74	78.22
Marjoram essential oil	92.73	93.75	100.00	100.00	97.83	95.05
Patchouli essential oil	96.36	100.00	100.00	100.00	100.00	98.02
Rosewood Oil	74.55	93.75	100.00	75.00	95.65	84.16
Sandalwood oil	94.55	100.00	92.31	100.00	95.65	95.05
Thyme oil	12.73	25.00	3.85	0.00	10.87	11.88
Zanthoxylum rhetsa essential oil	96.36	100.00	73.08	100.00	84.78	91.09
Multiple-Herbal drug-resistance index	0.58	0.61	0.56	0.50	0.57	0.58
Multiple drug resistance index	0.12	0.13	0.16	0.37	0.17	0.14
Multiple drug resistant strains	27	10	17	4	31	58
Extended spectrum β-lactamase producers	9	5	8	3	16	25

Table 3: Conventional antimicrobial resistance pattern of *Salmonella enterica* isolated from different sources.

Antimicrobials	**Percent resistant strains from different sources**					
	Reference (55)	***Salmonella* from Clinical samples from**				**Total (101)**
		Poultry birds (16)	**Domesticated animals (26)**	**Wild animals (4)**	**Total (46)**	
Amoxicillin	18.18	43.75	19.23	75.00	32.61	24.75
Amoxicillin + clavulanic acid	18.18	31.25	7.69	75.00	21.74	19.80
Ampicillin	5.45	18.75	42.31	100.00	39.13	20.79
Azithromycin	70.91	100.00	100.00	100.00	100.00	84.16
Aztreonam	0.00	0.00	7.69	25.00	6.52	2.97
Cefepime	0.00	0.00	3.85	0.00	2.17	0.99
Cefotaxime	0.00	6.25	7.69	25.00	8.70	3.96
Cefotaxime + clavulanic acid	0.00	6.25	3.85	25.00	6.52	2.97
Cefoxitin	1.82	18.75	19.23	50.00	21.74	10.89
Ceftazidime	23.64	0.00	7.69	50.00	8.70	16.83
Ceftriaxone	0.00	0.00	3.85	25.00	4.35	1.98
Chloramphenicol	5.45	0.00	7.69	0.00	4.35	4.95
Enrofloxacin	5.45	12.50	15.38	75.00	19.57	11.88
Colistin	25.45	6.25	11.54	0.00	8.70	17.82
Cotrimoxazole	21.82	0.00	11.54	0.00	6.52	14.85
Gentamicin	9.09	6.25	3.85	0.00	4.35	6.93
Imipenem	0.00	0.00	0.00	25.00	2.17	0.99
Meropenem	0.00	0.00	0.00	25.00	2.17	0.99
Moxalactam	0.00	6.25	0.00	0.00	2.17	0.99
Nalidixic acid	30.91	31.25	30.77	75.00	34.78	32.67
Nitrofurantoin	16.36	0.00	26.92	25.00	17.39	16.83
Piperacillin	7.27	25.00	19.23	50.00	23.91	14.85
Piperacillin + Tazobactam	1.82	0.00	15.38	25.00	10.87	5.94
Tetracycline	32.73	18.75	23.08	50.00	23.91	28.71
Tigecycline	3.64	0.00	0.00	25.00	2.17	2.97

Table 4: Herbal antimicrobial drug resistance pattern and other antimicrobial resistance traits of *Salmonella* strains of different origin associated with different ailments.

Antimicrobials and Resistance Measures	Resistance Pattern of *Salmonella* Isolates Associated with Different Ailments					
	Abortion (9)	Abscess (1)	Death (21)	Diarrhoea (13)	Nasal Catarrh (1)	Post surgery wound infection (1)
Agar wood Oil (AWO)	9	1	21	13	1	1
Ajowan oil (AO)	0	0	4	0	0	0
Betel leaf oil (BLO)	8	0	12	11	1	0
Carvacrol	0	0	3	0	0	0
Cinnamaldehyde	0	0	1	0	0	0
Cinnamon oil (CO)	0	0	2	1	0	0
Citral	6	1	14	9	1	1
Guggul oil (GO)	9	1	18	13	1	1
Holy basil oil (HBO)	0	0	0	2	0	0
Lemongrass oil (LGO)	6	0	17	9	0	1
Marjoram essential oil (MEO)	9	1	20	13	1	1
Patchouli essential oil (PEO)	9	1	21	13	1	1
Rosewood Oil (RWO)	9	1	19	13	1	1
Sandalwood oil (SWO)	8	0	21	13	1	1
Thyme oil (TO)	0	0	3	1	1	0
Zanthoxylum rhetsa essential oil (ZEO)	6	1	21	9	1	1
MHARI	4.9375	0.4375	12.3125	7.5	0.625	0.5625
MRI	1.44	0.08	3.56	1.8	0.32	0.44
MDR	8	0	12	9	1	1
Total ESBL	1	0	11	2	1	1

Table 5: Antimicrobial drug resistance pattern of *Salmonella* strains of different origin associated with different ailments.

Antimicrobials	Resistance Pattern of *Salmonella* Isolates Associated with Different Ailments					
	Abortion (9)	Abscess (1)	Death (21)	Diarrhoea (13)	Nasal Catarrh (1)	Post surgery wound infection (1)
Amoxicillin	1	0	7	5	1	1
Amoxicillin + clavulanic acid	0	0	3	5	1	1
Ampicillin	2	0	10	4	1	1
Azithromycin	9	1	21	13	1	1
Aztreonam	1	1	1	0	0	0
Cefepime	0	0	0	0	0	1
Cefotaxime	2	0	2	0	0	0
Cefotaxime + clavulanic acid	1	0	2	0	0	0
Cefoxitin	2	0	7	0	0	1
Ceftazidime	0	0	1	3	0	0
Ceftriaxone	1	0	1	0	0	0
Chloramphenicol	1	0	0	0	0	1
Enrofloxacin	0	0	7	1	0	1
Colistin	0	0	1	2	1	0
Cotrimoxazole	0	0	1	0	1	1
Gentamicin	1	0	1	0	0	0
Imipenem	0	0	1	0	0	0
Meropenem	0	0	1	0	0	0
Moxalactam	0	0	1	0	0	0
Nalidixic acid	3	0	10	1	1	1
Nitrofurantoin	2	0	1	4	1	0
Piperacillin	5	0	2	4	0	0
Piperacillin + Tazobactam	4	0	1	0	0	0
Tetracycline	1	0	7	2	0	1
Tigecycline	0	0	0	1	0	0

Table 6: Pearson correlation coefficient based significant ($p \leq 0.05$) negative and positive correlation in *Salmonella* inhibition zones induced by different herbal and conventional antimicrobials.

Herbal antimicrobial	Positive correlation in diameter of growth inhibition zone of *Salmonella* strains	Negative correlation in diameter of growth inhibition zone of *Salmonella* strains
Agarwood Oil (AWO)	Cinnamaldehyde, CO, GO, LGO, PEO	BLO, HBO
Ajowan oil (AO)	BLO, carvacrol , CO , citral, GO, RWO, SWO, TO, amoxicillin + clavulanic acid, amoxicillin, aztreonam, ceftriaxone, colistin, cotrimoxazole, imipenem, nalidixic acid, nitrofurantoin, iperacillin tigecycline	
Betel leaf oil (BLO)	AO, CO, carvacrol , HBO, SWO, TO, amoxicillin + clavulanic acid, aztreonam, colistin, moxalactam, nalidixic acid, piperacillin, piperacillin + tazobactam, tetracycline, tigecycline	AWO, azithromycin, ceftazidime
Carvacrol	AO, BLO, cinnamledehyde, CO , TO, nalidixic acid, nitrofurantoin, imipenem	GO, azithromycin, cefepime, gentamicin
Cinnamaldehyde	AO, carvacrol , CO , PEO, TO, colistin, nalidixic acid, nitrofurantoin, tigecycline	
Cinnamon oil (CO)	AO, AWO, BLO, carvacrol , cinnamaldehyde, PEO, TO, amoxicillin + clavulanic acid, cefotaxime, ceftriaxone, colistin, imipenem, nalidixic acid, nitrofurantoin, piperacillin, tigecycline	
Citral	AO, GO, PEO, amoxicillin, aztreonam, cefepime, ceftriaxone, enrofloxacin, colistin, imipenem, moxalactam, nalidixic acid, piperacillin, tigecycline	
Guggul oil (GO)	AO, AWO, citral , LGO, PEO, azithromycin, cefepime, cefotaxime + clavulanic acid, gentamicin, meropenem, moxalactam	Carvacrol
Holy basil oil (HBO)	BO, MEO, amoxicillin + clavulanic acid, piperacillin+ tazobactam, tigecycline	AWO, PEO, ceftazidime
Lemongrass oil (LGO)	AO, GO, PEO, cefotaxime+ clavulanic acid, nalidixic acid	Ampicillin
Marjoram essential oil (MEO)	HBO, piperacillin + tazobactam	Enrofloxacin
Patchouli essential oil (PEO)	AWO, cinnamaldehyde, CO , citral , GO, LGO, SWO	HBO, cotrimoxazole
Rosewood Oil (RWO)	Amoxicillin + clavulanic acid, azithromycin, ceftriaxone, colistin, cotrimoxazole, gentamicin, nitrofurantoin	Cefotaxime+ clavulanic acid, moxalactam
Sandalwood oil (SWO)	AO, BLO, PEO	
Thyme oil (TO)	AO, BLO, carvacrol , cinnamaldehyde, CO, ceftazidime, ceftriaxone, colistin, imipenem, nitrofurantoin, tigecycline	
Zanthoxylum rhetsa essential Oil (ZEO)		Nitrofurantoin

sensitive to GO than *S.* Kentucky, *S.* Paratyphi A, *S.* Pullorum, *S.* Typhi, *S.* Typhimurium and *S.* Virchow.

Strains of *S.* Paratyphi A and *S.* Pullorum were more often ($p < 0.05$) sensitive to BLO, PEO and SWO than *S.* Abortusequi, *S.* Kentucky, *S.* Typhimurium, *S.* Virchow. Isolates of *S.* Typhi were more ($p < 0.05$) commonly sensitive to nalidixic acid than *S.* Typimurium and *S.* Virchow strains.

Repository *S.* Typhimurium strains were more often ($p < 0.05$) resistant to ampicillin and RWO but less often ($p < 0.05$) to azithromycin, BLO and cefoxitin than those *S.* Typimurium isolated from clinical samples.

DISCUSSION

In the study, 57.4% salmonellae had MDR and 24.6% produced ESBL. However, it may not be considered as a novel finding as emergence of MDR *Salmonella* is being reported worldwide [7,12]. Occurrence of MDR *Salmonella* in animals is largely thought be due to use of antimicrobials as prophylactics and growth promoters [11]. The only isolate (*S. salamae*) resistant to carbapenems was from a Himalayan beer indicating rarity of carbapenem resistance in salmonellae. All the four isolates from wild animals had MDR and three of them produced ESBL. In the study >75% salmonellae were susceptible to most of the antibiotics except tetracycline, nalidixic acid and azithromycin. Tetracycline is one of the most commonly used antibiotics in animals and resistance might be associated with frequent use [11] but many of the antibiotics which failed to inhibit several strains of salmonellae in the study including azithromycin, meropenem, imipenem, moxalactam, cefepime, ceftriaxone, tigecycline, aztreonam and colistin are either not used or rarely used or prohibited for use in animals [21,22]. The occurrence of resistance in isolates of salmonellae towards non-animal-use antibiotics and in isolates from wild life, away from any antimicrobial use, indicated that it is not necessary that the antibiotic should be used in the target animals for occurrence of drug resistant strains and antimicrobial resistant (AMR) bacteria might be circulating in the environment affecting birds, humans, domesticated and wild animals as well [23]. Widespread occurrence of MDR bacteria in captive wild life is also reported earlier [24]. The emergence of MDR in wild animals clearly indicated that it is not only the direct antibiotic load in a particular species but a total environmental load of antibiotics may be responsible for global emergence of the MDR strains of bacteria [24,25].

None of the antibiotic or herbal antimicrobial inhibited all the 101 salmonellae tested. It indicated a wide variation in antimicrobial resistance patterns and probably multiplicity of resistance (R) factors (genes) carried by salmonellae [23]. However, to determine types of R-factors prevalent in *Salmonella* of animal origin more studies are required.

Among herbal antimicrobials, cinamaldehyde inhibited 95 strains closely followed by carvacrol, AO, CO, TO and HBO inhibiting 94, 93, 91, 89 and 87 strains, respectively. Though it appeared that there were six potential herbals inhibiting salmonellae, in reality they were only three, 1) cinnamon is the origin for cinnamaldehyde, an active ingredient of cinnamon oil, 2) carvacrol is the active ingredient in AO and TO and 3) HBO containing eugenol. These three groups of herbs have frequently been reported effective against several pathogenic bacteria from food, environment, animals and birds [13,26,27]. Of the 16 tested herbal antimicrobials, six were quite good in inhibiting salmonellae while a set of six herbals was almost useless inhibiting <10% of the isolates. Herbal antimicrobials including AWO, PEO, MEO, SWO, GO and ZEO restricted growth of 1, 2, 5, 5, 8 and 9 strains of *Salmonella*, respectively at concentration of 1µL/ per disc. The remaining four herbals including citral, BLO, LGO, and RWO failed to inhibit 66 (65.35%), 69 (68.32%) 79 (78.22%), and 85 (84.16%) salmonellae, respectively. In earlier studies too [15,16] these herbs have been reported effective only against a few salmonellae. The observations are in concurrence to earlier studies [21,28-30]. In an earlier study on 56 isolates of salmonellae of environmental and food origin, LGO has been reported to inhibit only one isolate [20] and majority of salmonellae from house gecko (*S. indica* 30, *S. salamae* 7 and *S. houtenae* 4) were resistant to essential oils of *Ageratum conyzoides, Artemisia vulgaris*, LGO, PEO, SWO and ZEO [28,29]. In other study on strains of *S.* Abortusequi (5), *S.* Adelaide (2), *S.* Javiana (1), *S.* Typhi (1), *S.* Anatum (1), *S.* Deversoir (1), *S.* Gallinarum (1), *S.* Kentucky (10), *S.* Typhimurium (3), *S. salamae* (11) isolated from different clinical sources, most of the isolates were resistant LGO, SWO, PEO and AWO [30].

Of the 12 *S.* Kentucky isolates tested, three were sensitive to ZEO but none to PEO. In an earlier study too on diarrhoeal isolates of salmonellae none of 7 *S.* Kentucky and one *S.* Typhimurium was inhibited by PEO but 4 of these were sensitive to ZEO [27]. The variation observed might be due to difference in source of isolation of the strains used in the two studies. The high frequency of resistance among *Salmonella* for many of the herbal antimicrobials might be due to their inherent resistance or due to requirement of higher inhibitory concentrations of the antimicrobials used in the present study. Three of the five *S.* Gallinarum were sensitive to GO but none of the six *S.* Pullorum strains and majority of the strains of other serovars was resistant to GO. However, in earlier studies [31] all *S.* Gallinarum were reported to be resistant to guggul gum (5 mg/ disc), it might be due to less concentration of GO in guggul gum.

Variation observed in antimicrobial activity of herbs among salmonellae tested than reported earlier in different studies might be due to several reasons including varietal difference of herb, time of harvesting, place of origin, part of the herb used, method of extraction of active ingredient [32] and bacterial strains tested [33]. Ethanolic extract of *Eupatorium triplinerve* inhibited *S.* Typhi and *S.* Paratyphi at 1 mg/mL concentration [34] but aqueous extract from leaves of *Eupatorium odoratum* failed to inhibit any and methanolic extract inhibited one *S. houtenae* and nine of the 40 *S. indica* strains [33].

The study observed no correlation between MAR and HMAR, and MRI and MHRI. It indicated that herbal antimicrobials might be working in independent pattern than that of antibiotics; and herbal antimicrobials may not be an answer to MDR strains. But significant negative correlation was evident among zones of inhibition (ZIs) produced by agar wood oil with ZIs induced by BLO and HBO; among ZIs of PEO and HBO, and among ZIs of Go and carvacrol. It indicated the probable way to create combinations of herbs to increase their spectrum of activity. The ZIs of azithromycin, ceftazidime and enrofloxacin against salmoeallae had significant ($p \leq 0.05$) negative correlation with ZIs induced by BLO and carvacrol, among ZIs of BLO and HBO. The observations indicated difference in mechanism of action of different herbal antimicrobials and variation in susceptibility of salmonellae to these compounds. The widespread resistance to different antimicrobials among different salmonellae serovars indicated that herbal drug resistance (HDR) is also matter of time and if herbal antimicrobial load may increase in the environment as a result of their excessive and regular use HDR may also be more common as suggested earlier [35,36].

The study indicated efficacy of several herbal compounds including cinnamaldehyde (in cinnamon, camphor and cassia oils), carvacrol (in oregano, thyme, thymus and ajowan oils) and eugenol (in holy basil oil) inhibit salmonellae. However, to use the potential herb(s) in therapeutics is a big challenge. It is because of several reason associated with ill understood biological, chemical and pharmaceutical properties of herbs. Limited long-term toxicity studies, poor understanding of pharmacodynamics and pharmacokinetics, and lack of verifiable clinical trials data (at various stages of drug development), problems of quality control in lack of standard testing and preparation protocols, reference values and pharmacopoeia are some of the limiting factors. Thus, using herbal antibacterial as future drugs either as an alternative or as a complementary therapeutic agent is still farfetched dream to come true [13,14].

The study concluded that on animal and poultry salmonellae, tetracycline, one of the most commonly used antibiotic in veterinary practice, may be useless now-a-days. However cefalosporins and quinolones still hold ground for their use in veterinary therapeutics for treating salmonellosis. Among herbal antimicrobials, herbs containing carvacrol, cinnamaldehyde and to some extent eugenol may be explored for development of useful therapeutic preparations to curtail growth of salmonellae.

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REFERENCES

1. CDC. National Antimicrobial Resistance Monitoring System for enteric bacteria (NARMS) annual reports and interactive data. Atlanta, GA: US Department of Health and Human Services, CDC. 2016.
2. Barrow PA, Methner U. *Salmonella* in domestic animals. 2nd ed. Germany: CABI; 2013. https://tinyurl.com/y8vmd36a
3. Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology. 9th ed. 1994. https://tinyurl.com/yctkm436
4. Verma JC, Singh VP, Singh BR, Gupta BR. Occurrence of *Salmonella* serotypes in animals in India VII. Indian J Comp Microbiol Immuol Infect Dis. 2001; 22: 51-55. https://tinyurl.com/ycotath4
5. Popoff MY, Le Minor L. Antigenic formulas of the *Salmonella* serovars. World Health Organization collaborating centre for reference and research on *Salmonella*. Pasteur Institute, Paris, France. 1997.
6. Makendi C, Page AJ, Wren BW, Le Thi Phuong T, Clare S, Hale C, et al. A phylogenetic and phenotypic analysis of *Salmonella enterica* serovar Weltevreden, an emerging agent of diarrheal disease in tropical regions. PLOS Neg Trop Dis. 2016; 10: e0004446. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/26867150
7. Gong J, Kelly P, Wang C. Prevalence and antimicrobial resistance of *Salmonella enterica* serovar Indiana in China (1984–2016). Zoonoses

Public Health. 2017; 64: 239-251. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/28009105

8. Gong J, Zeng X, Zhang P, Zhang D, Wang C, Lin J. Characterization of the emerging multidrug-resistant *Salmonella enterica* serovar Indiana strains in China. Emerg Microbes Infect. 2019; 8: 29-39. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/30866757

9. Wiedemann A, Virlogeux-Payant I, Chaussé AM, Schikora A, Velge P. Interactions of *Salmonella* with animals and plants. Front Microbiol. 2015; 5: 791. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/25653644 10. Demirbilek SK. Salmonellosis in animals. 2017. https://tinyurl.com/ycgoray3

10. Kuehn B. Multidrug-resistant *Salmonella*. JAMA. 2019; 322: 1344. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/31593279

11. Chatham-Stephens K, Medalla F, Hughes M, Appiah GD, Aubert RD, et al. Emergence of extensively drug-resistant *Salmonella* Typhi infections among travelers to or from Pakistan — United States, 2016–2018. MMWR Morb Mortal Wkly Rep. 2019; 68: 11-13. **PubMed**: https://www.ncbi.nlm.nih.gov/pubmed/30629573

12. Bhardwaj M, Singh BR, Sinha DK, Vadhana P, Vinodhkumar, Varan Singh S, et al. Potential of herbal drug and antibiotic combination therapy: A new approach to treat multidrug resistant bacteria. Pharm Anal Acta. 2016; 7: 11. https://tinyurl.com/y7juepvv

13. Vinodh, Kumar O.R, Singh B.R, Sinha K, Dubal Z.B, Pruthvishree B.S, et al. Tackling antimicrobial resistance: Current approaches. J Immunol Immunopathol. 2019; 21: 1-9. https://tinyurl.com/yctjxnaw

14. Dougnon TV, Déguénon E, Fah L, Lègba B, Hounmanou YMG, Agbankpè J et al. Traditional treatment of human and animal salmonelloses in Southern Benin: Knowledge of farmers and traditherapists. Vet World. 2017; 10: 580-592. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/28717307

15. Lee MH, Kwon HA, Kwon DY, Park H, Sohn DH, Kim YC, et al. Antibacterial activity of medicinal herb extracts against Salmonella. Int J Food Microbiol. 2006; 111: 270-275. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/16876280

16. Carter G R. Diagnostic Procedures in Veterinary Microbiology. 2nd ed. Springfield: Charles C Thomas Publishers. 1975. https://tinyurl.com/ycgv2cc8

17. Singh BR. Labtop for Microbiology Laboratory. Germany. Lambert Academic Publishing. 2009. https://tinyurl.com/yay7ohnb

18. Performance standards for antimicrobial disk susceptibility tests, 24th Informational Supplement, Document M100-S24 and M11-A8. Clinical and Laboratory Standards Institute*,* Wayne, Pennsylvania. 2014.

19. Singh BR, Singh V, Singh RK, Ebibeni N. Antimicrobial activity of lemongrass (*Cymbopogon citratus*) oil against microbes of environmental, clinical and food origin. Int Res J Pharmacy Pharmacol. 2011; 1: 228-236. https://tinyurl.com/y7b3ys74

20. Singh BR, Somvanshi R. Antimicrobial sensitivity assay and antimicrobial chemotherapy in Animals: A Practical approach. In: Diseases of Animals: Diagnosis and Management. Indian Veterinary Research Institute, Izatnagar. 2013; 7-31. https://tinyurl.com/yd76765h

21. Singh BR. Guidelines for antimicrobial drug use in animals, Technical Report IVRI-5. Indian Veterinary Research Institute, Izatnagar. 2020. https://tinyurl.com/y9jbu3d8

22. Kumar S, Singh BR. An overview on mechanisms and emergence of antimicrobials drug resistance. Ad Anim Vet Sci. 2013; 1: 7-14. https://tinyurl.com/ycjf2q9a

23. Singh BR. Emergence of antibiotic resistance in captive wildlife. In: National congress on wildlife health and annual convention of Association of Indian Zoo and Wildlife Veterinarians (AIZWV). ICAR-Indian Veterinary Research Institute, Izatnagar. 2017; 4-9. https://tinyurl.com/ybz2lwav

24. Singh BR. Who is responsible for AMR and how to handle it? 17th Annual Convocation cum National Seminar on "Livestock Sector towards One Health, Food Security and Safety" during 19th to 20th December 2018. College of Veterinary Science and Animal Husbandry Orissa University of Agriculture and Technology, Bhubaneswar-751003, Odisha, India. 2018. https://tinyurl.com/y7cr3ax2

25. Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali SM, et al. Antimicrobial activity of five herbal extracts against Multi Drug Resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules. 2009; 14: 586-597. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/19214149

26. Singh BR. Vinodh Kumar OR, Sinha DK, Bhardwaj M, Saraf A, et al. Antimicrobial resistance profile of enteropathogens isolated from diarrhea patients: Herbal antimicrobials, a ray of hope. Annals Pharmacol Pharmaceut. Ann Pharmacol Pharmaceut. 2017; 2: 1068-1078. https://tinyurl.com/ya7mdek7

27. Singh BR, Singh V, Ebibeni N, Singh RK. Antimicrobial and herbal drug resistance in enteric bacteria isolated from faecal droppings of common house lizard/gecko (*Hemidactylus frenatus*). Int J Microbiol. 2013; 8. https://tinyurl.com/yc2m8z8n

28. Singh BR, Sinha DK, Vinodh Kumar OR, Pawde AM, De UK, Vinod Gupta. Comparative antimicrobial activity of *Pogostemon cablin* (patchouli) Essential Oil (PEO) and conventional antimicrobials against clinically important microbes. World J Pharm Sci. 2019; 7: 47-65. https://tinyurl.com/y8zjodlm

29. Singh BR, Sinha DK, Vinodhkumar OR. Effect of herbal antimicrobials on bacterial strains of foods of vegetable and animal origin. J Food Chem Nanotechnol. 2016; 2: 115-123. https://tinyurl.com/y8pj2ar2

30. Singh BR, Siddiqui MZ. Antimicrobial activity of *Commiphora wightii* gum (Guggul gum) extract against gram positive and gram negative bacteria. J Microbiol Antimicrob Agents. 2015; 1: 36-39. https://tinyurl.com/y96mfwkl

31. Usman JG, Sodipo OA, Sandabe UK. *In vitro* antimicrobial activity of *Cucumis metuliferus* E. Mey. Ex. Naudin fruit extracts against *Salmonella gallinarum*. Int J Phytomed. 2014; 6: 268-274. https://tinyurl.com/yaneds7u

32. Singh BR, Agrawal RK, Sinha D.K, Vinodhkumar O.R., Vadhana P, Monika Bhardwaj, et al. Antimicrobial activity in aqueous and methanolic leaf extract of *Eupatorium odoratum* against bacteria of clinical and nonclinical origin. J Adv Clin Pharmacol. 2015; 2: 50-59. https://tinyurl.com/yblyjuay

33. Rahman MS, Junaid M. Antimicrobial activity of leaf extracts of *Eupatorium triplinerve* Vehl against some human pathogenic bacteria and phytopathogenic fungi. Bangladesh J Bot. 2008; 37: 89-92. https://tinyurl.com/y8hcse95

34. Vadhana P, Singh BR, Bhardwaj M, Singh SV. Emergence of herbal antimicrobial drug resistance in clinical bacterial isolates. Pharm Anal Acta. 2015; 6: 10. https://tinyurl.com/y8n8geuq

35. Singh BR, Sinha DK, Vinodh Kumar OR, Vadhana P, Bhardwaj M, Saraf A, et al. Antimicrobial activity of agarwood oil against Multiple-Drug-Resistant (MDR) microbes of clinical, food and environmental origin. Curr Drug Discov Technol. 2019. **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed/30686261