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SYNERGISM BETWEEN *MIMUSOPS ELENGI* AND *BAUHINIA VARIEGATA* SEED EXTRACTS AGAINST *SALMONELLA ENTERICA* SEROVAR TYPHI AND *VIBRIO CHOLERAE* O1 BIOTYPE EL TOR SEROTYPE OGAWA ISOLATES

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ABSTRACT
The current communication evaluates the antibacterial activity of *Mimusops elengi* (*M. elengi*) and *Bauhinia variegata* (*B. variegata*) seed extracts, alone and in combination, against *Salmonella enterica* serovar Typhi (*S. typhi*) and *Vibrio cholerae* O1 biotype El Tor serotype Ogawa (*V. cholerae*) isolates. The antibacterial activity of ethanolic extracts of bakul, *M. elengi*, seed (MSE; 500 µg) and kanchan, *B. variegata*, seed (BSE; 500 µg), alone and in combination, was determined following agar diffusion method, for a total of 16 *S. typhi* and *V. cholerae* isolates. The zone diameter of inhibition (ZDI) for the agents was recorded, and growth inhibitory indices (GIIs) were calculated. The *V. cholerae* and *S.* Typhi isolates had BSE (500 µg) and MSE (500 µg) ZDIs 12-17 mm and 13-15 mm, respectively. The GIIs of the BSE-MSE combination ranged 0.654 - 0.788 and 0.538 - 0.759 for the isolates of *S. typhi* and *V. cholerae*, respectively. The combined activity of BSE and MSE was synergistic against the test bacterial isolates, and the test plant extracts are potential in combating *S. typhi* and *V. cholerae* drug resistance and hence are important sources for the development of non-antibiotic drug(s) against *S. typhi* and *V. cholerae* infection.

KEY WORDS: Antibacterial activity, *Mimusops elengi*, *Bauhinia variegata*, Zone diameter of inhibition, Growth inhibitory index, Synergy, *S.* Typhi, *V. cholerae* Ogawa

INTRODUCTION

The two life threatening infectious diseases, typhoid and cholera, caused with the infection of *Salmonella enterica* serovar Typhi (*S.* typhi) and *Vibrio cholerae* (*V. cholerae*), respectively, are endemic in India; the emergence and prevalence of the micro-pathogens having resistance to multiple antibiotics are reported worldwide. The development of multi-drug resistances among the disease causing bacteria prompted the scientists in the field to discover new sources of non-antibiotic drugs in various plants, in order to tackle the disadvantages of antibiotic resistance. The *in vitro* antibacterial activity of *Camellia sinensis* ethanolic extract against multidrug-resistant (MDR) clinical isolates of *S.* Typhi and *V. cholerae* O1 biotype El Tor serotype Ogawa (*V. cholerae* Ogawa) has been demonstrated earlier[1]. The growth inhibition activity of the extracts of different parts - bark, fruit and seed of *Mimusops elengi* L. (family: Sapotaceae) against different bacteria has been reported[2]. Hazra *et al.*[3] reported the antibacterial activity of *M. elengi* seed extract. Among the different solvent extract tested, as has been reported by Lalitha *et al.*[4], the methanol and ethanol extract showed activity against *Escherichia coli*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *S.* Typhi, *V. cholera* and *Streptococcus pneumonia* (*Str. pneumonia*). The ethyl acetate, ethanol and methanol extracts of *M. elengi* showed antibacterial activity against dental caries causing bacteria *Str. mutans*[5]. The plants of the genus *Bauhinia* have a long history of traditional medicinal applications[6], and the medicinal use of *B. variegata* as an anti-infectious diseases, as a natural sources, has been justified[7]. The antimicrobial activity of different extracts of *B. variegata* bark has been reported against different gram-positive and gram-negative bacteria[8]. The ethanol extract of *B. variegata* leaves showed inhibitory activity against *S. typh*i, *V. cholera*, *Klebsiella pneumoniae*, *E. coli* and *Staphylococcus aureus* (*Staph. aureus*)[9]. Herein, we assess the antibacterial activity of ethanolic *M. elengi* and *B. variegata* seed extracts, alone and in combination, against multi-drug resistant (MDR) clinical *S. typhi* and *V. cholerae* Ogawa isolates.

MATERIALS AND METHODS

Bacterial isolates

The MDR blood culture isolates of *S. Typhi* (n=8) and rectal swab culture isolates of *V. cholerae* Ogawa (n=8), respectively from typhoid patients and cholera cases, were selected for the present study carried out partly at the Calcutta School of Tropical Medicine, India and partly at the KPC Medical College, Kolkata, India. The control strain was *Escherichia coli* ATCC 25922.

Plant materials and extract preparation

The fully matured seeds of kanchan (*B. variegata*) were collected from the Subhas Pally Jr. High School campus, Kolkata, India, while the seeds of bakul (*M. elengi*) were taken out from the ripen fruits collected around the localities of the above mentioned school and from the Gurudas College campus, Kolkata, India. Both types of

seeds were sun-dried and the extracts were prepared following the protocol published earlier[1], using 50 g of the dried materials, and ethanol as the extractant. The *B. variegata* seed extract (BSE) and *M. elengi* seed extract (MSE) obtained were stored at 4°C in 50 % ethanol (final concentration of 10 µg/mL), and were utilized within one week; the extracts were prepared freshly when needed.

Agar diffusion susceptibility

The antibacterial activity of BSE and MSE was assessed for *S. typhi* (n=8) and *V. cholerae* Ogawa (n=8) isolates following agar diffusion technique, on Mueller- Hinton agar (MHA) plates, each of which was inoculated with 10^8 CFU. The test protocol has been described elsewhere[10]. Briefly, each of the plates were divided into three equal sectors and marked with BSE, MSE and BSE + MSE. The extracts, BSE and MSE (each 500 µg, *i.e.*, 50 µL), alone was dropped on the properly marked sectors of the MHA plates. In order to determine the combined effect of the extracts, BSE (250 µg i.e., 25 µL) plus MSE (250 µg*i.e.*, 25 µL), were dropped on the sector marked with BSE + MSE. Ceftriaxone at 30-µg disc was used as an antibiotic reference standard. The sensitivity of the test bacterial isolates to the plant extracts were considered with zone diameter of inhibition (ZDI) ≥7 mm[10].

Interpretation of the results

The antibacterial activity of BSE and MSE was recorded by measuring the ZDI due to their action, alone or in combination, after 24 h incubation at 35 ^{0}C. The growth inhibitory indices (GIIs) were calculated by using the formula: [ZDI obtained in combined action of BSE and MSE / (total of ZDIs of the two agents in single action)][10]. The synergistic, additive and antagonistic activities, if any, in between the two of the antimicrobial agents were defined with GIIs > 0.5, 0.5 and < 0.5, respectively; the other details are mentioned elsewhere[10].

RESULTS

The antibacterial activity, in terms of ZDI, of BSE (500 µg) and MSE (500 µg) alone and in combination (BSE 250 µg plus MSE 250 µg), for the test *S. typhi* and *V. cholerae* Ogawa isolates is shown in Figure 1. The ZDIs obtained due to the action of BSE and MSE for *V. cholerae* Ogawa isolates ranged 13-15 mm, while 12-17 mm for *S. typhi* isolates. The ZDIs obtained from the BSE-MSE combined action were 15-22 mm and 17-23 mm, respectively for *V. cholerae* Ogawa and *S. typhi* isolates.

The GIIs from BSE-MSE combination for the isolates are represented in Figure 2. The overall GIIs were >0.5, and ranged 0.538 - 0.759 for *V. cholerae* Ogawa and 0.654 - 0.788 for *S. typhi* isolates.

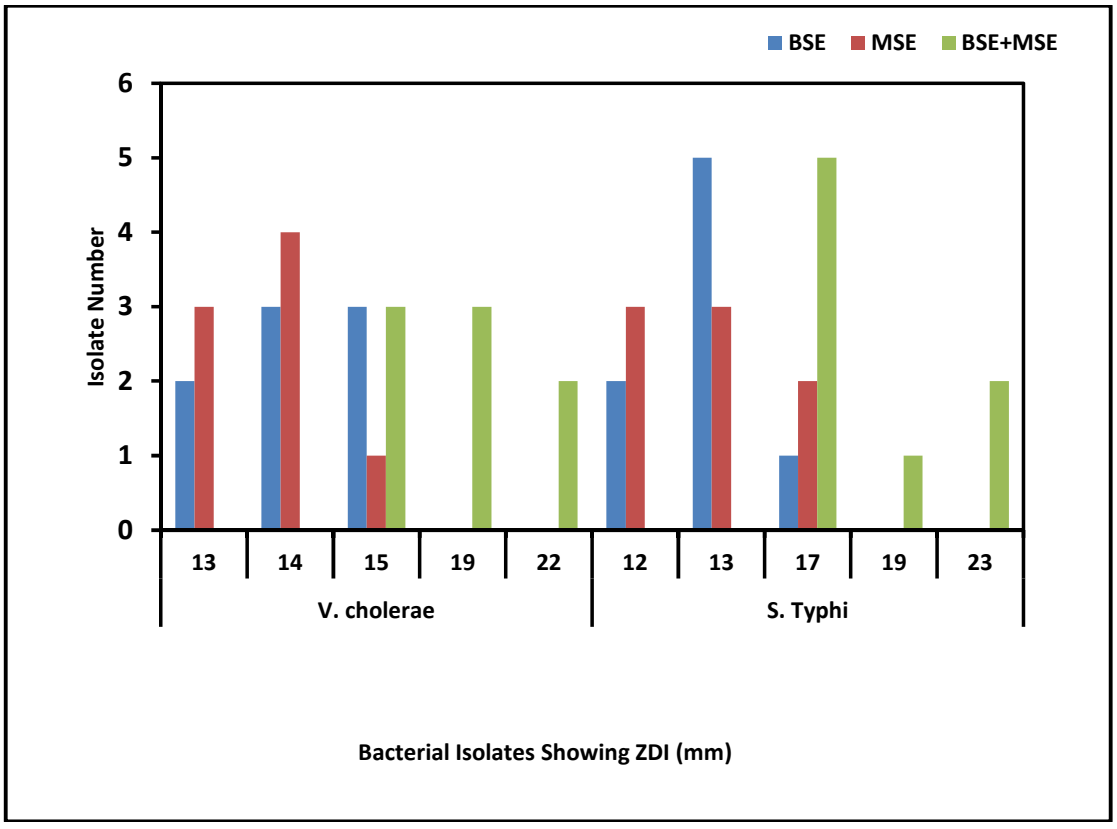

FIGURE 1: Zone diameter of inhibition (ZDI) of *M. elengi* seed ethanolic extract (MSE) and *B. variegata* seed ethanolic extract (BSE), alone and in combination, for *S. typhi* and *V. cholerae* Ogawa isolates

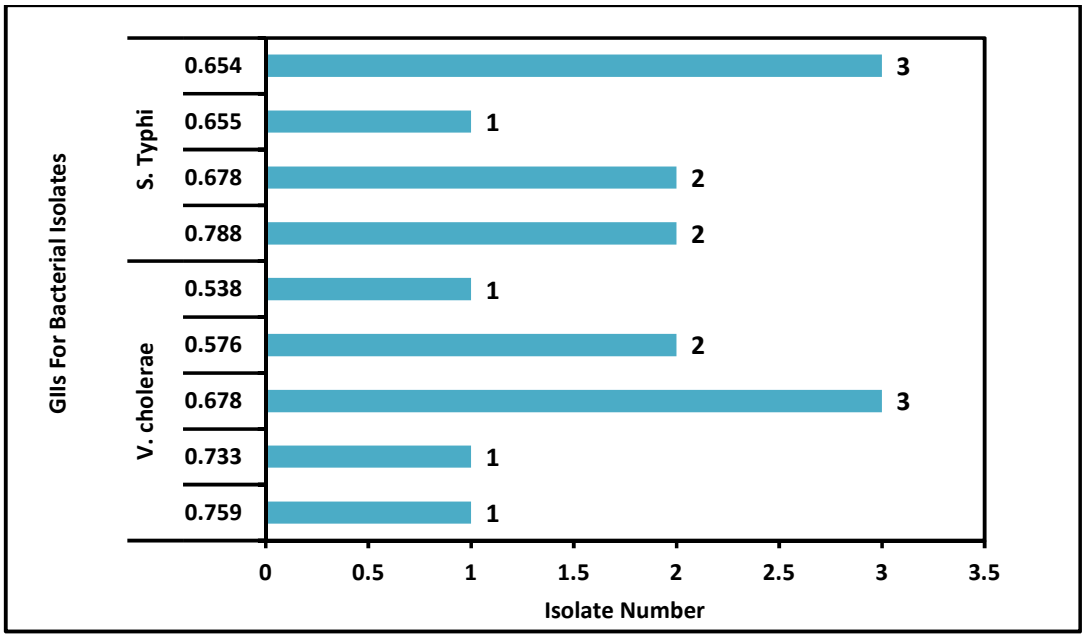

FIGURE 2: Growth inhibitory indices (GIIs) from combined action of MSE and BSE for *S. typhi* and *V. cholerae* Ogawa isolates. The abbreviations are mentioned in the Figure 1.

DISCUSSION

The plants are important source for the development of non-antibiotic drugs and the *in vitro* antibacterial test forms the basis of identifying the potential sources of such agents. The present communication explores the antibacterial activity of two plants: *M. elengi* (bakul) and *B. variegata* (Kanchan), based upon their traditional and medicinal uses in the remedies of various health disorders in humans (a large number of claims on wide range of folk curative properties of *B. vareigata* are recorded). Traditionally seeds of *M. elengi* are being used for curing piles, headache, constipation and such traditional claim are well supported by modern research[11,12]. The *M. elengi* leaf, bark, stem, fruit pulp, fruit rind, seed cotyledon seed testa, and flower extracts were tested at 20 mg/mL for potential antibacterial activity by agar diffusion methods against different clinical bacteria including *S. typhi*[12]. Sahu and Gupta[13] documented a detailed survey of the literature on pharmacognosy, phytochemistry, traditional medicinal uses and pharmacological activities of *B. variegata.*

M. elengi ethyl acetate extract showed potential inhibitory action against *Str. mutans* of 16 mm (5 mg/disc) and 14 mm (2.5 mg/disc)[5]. Lalitha *et al.*[4] reported the inhibitory activity of *M. elengi* against *V. cholerae* and *S.* Typhi having ZDIs 29.4 mm and 25.0 mm, respectively, in methanol extract (50 µL) and 20.9 and 20.8 mm, respectively, in ethanol extract (50 µL). Gunalan *et al.*[9] reported that *B. variegate* exhibited greater antibacterial activity against *S. typhi* (ZDI; 21 mm) followed by *V. cholera* (ZDI; 16 mm) at 500 µg of ethanolic leaf-extract. Gupta and Paarakh[14] reported that the petroleum ether, chloroform, methanol and aqueous extracts of *B. variegate* leaves were active against *Stap. aureus*, *Bacillus subtilis* (*B. subtilis*), *E. coli* and *P. aeruginosa*. Dhale[15] reported that the *B. variegata* leaf alcoholic extract (20mg/ml) exhibited activity against *Staph. aureus* (ZDI; 15 mm) *P. aeruginosa* (ZDI; 14 mm), *E. coli* (ZDI; 10 mm) and *B. subtilis* (ZDI; 9 mm), and the bark extracts of *B. variegata* (20mg/ml) exhibited activity against *Staph. aureus* (ZDI; 18 mm), *P. aeruginosa* (ZDI; 16 mm), *E. coli* (ZDI; 12 mm) and *B. subtilis* (ZDI; 10 mm). Ethanolic extract of *B. variegata* exhibited maximum inhibitory activity against *K. pneumonia*[7]. In the present study, BSE and MSE showed excellent activity against *V. cholerae* Ogawa isolates (ZDIs; 13-15 mm) and *S. typhi* isolates (12-17 mm); the ZDIs from the BSE-MSE combined action were 15-22 mm and 17-23 mm, respectively for *V. cholerae* Ogawa and *S. typhi* isolates. The earlier studies demonstrated synergistic activity of various plant extracts against different bacteria of clinical importance; however, the combined action of *M. elengi* and *B. variegata* has not been documented against *S. typhi* and *V. cholera*. The aqueous extracts of *Foeniculum vulgare* and ethanolic extracts of *Salvia officnalis* had 14 mm and 12 mm ZDIs, respectively, against *E. coli* O157:H7 in single action, while the *F. vulgare* aqueous extract in combination with *Priminella anisum* and *Carum carvi* showed 25 mm ZDI against *E. coli* O157:H7[16]. Combination of cumin and fenugreek aqueous extract showed synergistic activity against *Proteus vulgaris* (*Pr. vulgaris*) and additive effects against *Staph. aureus* and *B. cereus*, and black cumin and mustard demonstrated synergistic activity against *Staph. aureus* and additive effect against *S. enterica* and *Pr. vulgaris*[17]. The aqueous leaf extracts of *Cassia auriculata* L. (Fabaceae, Sub-family: Caesalpinoideae) and ethanolic leaf extracts of *Cissus quadrangularis* L. (Vitaceae) showed 18 mm and 17 mm ZDI, respectively against *E. coli* and *B. subtilis* while tested individually, and the combination of aqueous leaf extracts of *C. auriculata* and *C. quadrangularis* (1:1) showed 25 mm ZDI against *E. coli*; the highest ZDI (28 mm) was observed against *Staph. aureus* in combination of *Balanites aegyptiaca* and *Lobelia nicotianaefolia* [18].

Bulb and leaf extracts of three medicinal plants: *Tulbaghia violacea*, *Hypoxis hemerocallidea* and *Merwilla plumbea*, independently and in combinations, were assessed for antimicrobial activity against gram-positive and gram-negative bacteria; most extract combinations demonstrated either synergistic and additive/indifferent interaction effect against the test bacteria with only a few exhibiting antagonistic effects[19]. Berberine, an alkaloid from berberry plants, alone exhibited least antibacterial activity, but in combination with 5-methoxyhydnocarpin, produced by berberry plants, the agents exhibited potent antibacterial property[20,21]. Prakash *et al.*[22] reported that the ethanolic leaf extracts of *Catharanthus roseus*, *Lawsonia inermis* and *Chrysanthemum odoratum* showed poor activity against methicillin resistant *S. aureus* (MRSA) when used individually; the combination of *C. roseus* and *L. inermis* (23 mm), and *L. inermis* and *C. odoratum* (20 mm) exerted higher activity. The synergistic activity suggests different mode of action of the combining compounds, and hence effects supports the use of the plant extracts in combination instead of their use in isolation[23]. Moreover, the antimicrobial agents in combination are preferred as because the microbial resistance is less likely to develop against the components having different modes of action[24]. In the current study, the *M. elengi* and *B. variegata*, in combination had synergistic effect against *S. typhi* and *V. cholera* Ogawa. The greater ZDIs from the combined action of the test extracts compared to the ZDIs from their individual action, as well as the GII > 0.5 for the *S.* Typhi and *V. cholera* Ogawa isolates supported the fact[10,25]; the extracts in combination had no additive or antagonistic effects.

Many of the studies were useful in identifying the active principle responsible for such potentials and to develop clinically important therapeutic drugs for mankind.[3] Hazra *et al.* obtained two antibacterial compounds from the seeds of *M. elengi*: 2,3-dihyro-3,3’4’5,7-pentahydroxyflavone and 3,3’,4’,5,7-pentahydroxyflavone showing strong inhibitory activity against gram-positive and gram-negative bacteria. Phytochemical analysis of *M. elengi* revealed the presence of tanins, alkaloids, saponins, cardiac glycosides, steroids, flavonoids and reducing sugar[4]. *B. variegata* has been reported to be a potential source of phenols, tannins, flavonoids, steroids and of cardiac glycosides. The antibacterial activity of the test plant extracts might be due to the presence of phytochemicals as has been reported earlier; however, the bioactive compounds should further be evaluated for their antibacterial properties and should be subjected to *in vivo* trials, alone and in combination, before making treatment protocol against S. *typhi* and *V.* cholerae infection. The evidence of excellent *in vitro* activity of *M. elengi* and *B. variegata* ethanolic seed extracts and synergism between the two suggests their possible use as the cost effective anti-typhoid and anti-cholera drugs in treating human infection due to MDR *S.* Typhi and *V. cholerae*. The use of different parts of the two important Indian medicinal plants (*M. elengi* and *B. variegata*) in traditional medicine supports their safe use[26,27]; still future research is mandatory to determine the toxicity of the agents and/or the crude extracts in determining pharmacological dose for therapeutic application, alone or in combination.

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