

# *In Vitro* Antimicrobial Activity of Crude Leaf Extracts from *Aloe secundiflora*, *Bulbine frutescens*, *Vernonia lasiopos* and *Tagetes minuta* against *Salmonella typhi*

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## Abstract

Four medicinal plants leaves were investigated to evaluate their antibacterial potential of the methanol extracts against *Salmonella typhi* by disc diffusion method. The methanol extract from *Aloe secundiflora* showed strong antibacterial activity against a clinical isolate of *Salmonella typhi* at low concentrations (5.5 mg/ml) as compared to *Bulbine frutescens* extract (8.8 mg/ml). The minimum inhibitory concentration ranged from 5 mg/ml - 9 mg/ml whereas the maximum bactericidal concentration range from 7 mg/ml - 11 mg/ml. The standard antibiotic used was ciprofloxacin (15 µg/ml) was used as a positive control while dimethyl sulphoxide and distilled water were used as the negative control. The extracts were preliminary screened for the presence of secondary metabolites to determine the presence of flavonoids, alkaloids, tannins and saponins. The results supported the use of the medicinal plants in the treatment of infections caused by *Salmonella typhi*.

**Keywords:** *Salmonella typhi*; Disc diffusion; Medicinal plants; Secondary metabolites; Methanol extract

## Introduction

Medicinal plants have been identified and used throughout human history [1]. The use of medicinal plants (herbs) to treat diseases is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive conventional drugs [2]. The genus *Tagetes* belongs to the Asteraceae family which presently comprises of 56 species, 27 biennials and 29 perennials. *Tagetes* species are grown all over the world as multipurpose plants [3]. Plant parts such as flowers and leaves have been known to contain flavonoids that are scavengers for free radicals which enhances the antimicrobial activity of the *Tagetes minuta* extracts [4]. Phytochemicals from the plant such as carotenoids have also been used in pharmacological preparations and they have been found to contain anti-aging and anti-cancer effects [5]. The plant extracts have been used in treating intestinal and stomach problems [6,7]. Aloes are perennial succulent xerophytes which develops water storage tissues in leaves to survive in areas with low or erratic rainfall [8]. Aloe extracts have been used for many centuries for their curative and therapeutic properties [9]. Extracts of Aloes especially its leaf gel have shown antibacterial activity by inhibiting the growth of both Gram negative bacteria and Gram positive bacteria [10]. *Aloe secundiflora* leaf components have been credited for antibacterial, antifungal and antiviral and antihelminthic medicinal properties [11]. *Aloe secundiflora* has been used in treating ailments including; chest problems, polio, malaria and stomach ache by herbalists in the Lake Victoria region [12]. *Bulbine* is a genus of plants in the family xanthorrhoeaceae and sub family asphodeloideae and its members are well known for their medicinal value [13]. *Bulbine* plant has been used for medicinal purposes in the early stages of the eighteen century by Dutch and British settlers of South Africa in treating various ailments [14]. The leaves of the plant have been used in the treatment of wound thought to be infected with bacterial pathogens and it has shown antibacterial properties [15]. Some of the species of the plant found in South Africa have been used for blood cleansing, treatment of ringworms and gravel rush by some local communities such as the Xhosa [14]. A decoction of bulbs and roots of some of the species has been used in the treatment of some of the venereal diseases in women

and stomach upsets [16]. *Vernonieae* is a tribe which has about 1300 species and in the family Asteraceae (Compositae) which mostly contains herbaceous plants [17]. *Vernonia lasiopos* decoctions from the stems and leaves have been traditionally been used by herbalists in East Africa to treat, malaria, worms and gastrointestinal problems [18]. Its extracts have also been used in treating some of the sexually transmitted diseases in southern parts of Africa [19]. In this study the antimicrobial activity of the leaf extracts from the plants against clinical isolate of *Salmonella typhi* was tested and screened for the presence of phytochemicals.

## Materials and Methods

### Plant material collection

The fresh plant leaves of *Aloe secundiflora*, *Bulbine frutescens*, *Vernonia lasiopos* and *Tagetes minuta* were collected at Kenyatta University Arboretum. Voucher specimens were prepared and deposited in the university herbarium in Plant Sciences Department for future reference. The plants leaves were brought to the laboratory and thoroughly washed in running water to remove debris and dust particles and then rinsed using distilled water and finally air dried.

### Preparation of plant extract

The air dried plants leaves were tause into powder and soaked in methanol for 72 h and placed in a Gallenkamp shaker at 65 revolutions per minute. The contents were homogenized and filtered using

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Received January 27, 2016; Accepted February 09, 2016; Published February 15, 2016

Citation: Rachuonyo HO, Ogola PE, Arika WM, Nyamai DW, Wambani JR (2016) *In Vitro* Antimicrobial Activity of Crude Leaf Extracts from *Aloe secundiflora*, *Bulbine frutescens*, *Vernonia lasiopos* and *Tagetes minuta* against *Salmonella typhi*. J Tradi Med Clin Natur 5: 187. DOI: 10.4172/2573-4555.1000187

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whatman filter paper no. 1. The filtrate was poured into a round bottom flask and concentrated using a vacuum evaporator and stored in a labelled amber glass bottle at room temperature away from light and heat before being used for antibacterial efficacy test.

### Antimicrobial susceptibility testing

The microorganism used was clinical isolate of *Salmonella typhi* obtained from Kenyatta University Health Centre Laboratory, Nairobi. *Salmonella typhi* was tested against methanol extracts of *Tagetes minuta*, *Aloe secundiflora*, *Bulbine frutescens* and *Vernonia lasiopos*. *Salmonella typhi* inoculum was concentrated by comparing it with a 0.5 McFarland standard. Discs of 6 milliliters were prepared from whatman no.1 filter paper. The discs were sterilized by autoclaving. After sterilization the moisture discs were dried on hot air oven at 50°C [20]. The various solvent extracts discs prepared were impregnated with the extracts from the highest concentration of 1000 mg/ml to the lowest concentration of 1 mg/ml [21]. The antimicrobial efficacy test was carried out using Kirby Bauer method [22]. Muller Hinton agar was used in the spread plate technique where *Salmonella typhi* was spread using sterilized cotton wool swab and exposed to extracts impregnated discs in milligrams per microliter from *Aloe secundiflora*, *Tagetes minuta*, *Vernonia lasiopos* and *Bulbine frutescens*. The discs were placed with equal distance between them on agar plates inoculated with *Salmonella typhi*. Positive control discs contained ciprofloxacin (15 µg/ml) while negative control discs were impregnated with dimethyl sulphoxide and distilled water. The Petri dishes were incubated at 37°C for 24 h. Zones of inhibition formed were measured in millimetres and their average determined. The experiment was carried in duplicates and the diameter of zones of inhibition formed measured. Minimal Inhibitory Concentration (MIC) was determined using the broth tube method [23]. 100 µl of 250 mg/ml of methanol extract was added to 100 µl of sterile bacteriological peptone in the first well of the 96 well micro plates and mixed well with a micropipette. 100 µl of this dilution was transferred subsequently to wells two folding each dilution of the original extract. This was done to the extracts of *Aloe secundiflora*, *Bulbine frutescens*, *Vernonia lasiopos*, and *Tagetes minuta*. An inoculum of 100 µl (0.5 McFarland standards) of overnight clinical culture of *Salmonella typhi* was added in each of the wells. Triplicate of each micro plate were made and the procedure repeated. The plates were then incubated at 37°C for 24 h. After incubation 40 µl of 0.2 mg/µl of INT was added in each of the wells and the plates examined after an additional 60 minutes of incubation. Growth was indicated by a red colour (conversion of INT to formazan). The lowest concentration at which the colour was apparently invisible as compared to the next dilution was taken as the minimum inhibitory concentration [24]. Minimum bactericidal concentration (MBC) was determined by taking 100 µl of suspension from micro plate wells that demonstrated no growth and inoculated on agar plates. The plates were incubated at 37°C for 24 h. In the case where, there was no bacterial growth and value not greater than the minimum inhibitory concentration the concentration was used as the maximum bacterial concentration.

### Phytochemical analysis

Presence of saponins, tannins, flavonoids and alkaloids in the crude extract were determined [25].

**Tannins:** Each of the extracts was weighed to 0.5 mg and dissolved in 1 ml of distilled water. Filtration was carried out after 2 ml of FeCl<sub>3</sub> was added. If there was presence of a blue or black precipitate then it indicated the presence of tannins.

**Flavonoids:** Each of the extracts was weighed to 0.5 mg and dissolved in 1 ml of ethanol and filtered. 2 ml of 1% HCl and magnesium ribbon was added to the filtrate. If there was formation of a pink or red colour it indicated the presence flavonoids.

**Alkaloids:** Each of the extracts was weighed to 0.5 mg and dissolved in 1 ml of methanol and filtered. 1% HCL was added to the filtrate and the solution heated. Mayor`s reagent was added drop wise and if there was formation of any colored precipitate it indicated the presence of alkaloids.

**Saponins:** Each of the extracts was weighed to 0.5 mg and dissolved in 1 ml of methanol and filtered. Distilled water was added and shaking done for a few minutes. If there was persistence frothing then it indicated the presence of saponins.

### Results and Discussion

In this study the antibacterial activity of leaf extracts from *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopos* and *Bulbine frutescens* were evaluated against *Salmonella typhi*. Following methanolic extraction, the antibacterial activity of the extracts was determined by disc diffusion method. Table 1 presents the antimicrobial test of the plant extracts against *Salmonella typhi*. The results indicated that all the plants extracts were active against *Salmonella typhi*. The antimicrobial activity of these extracts varied greatly on Muller Hinton agar. The positive control used (Ciprofloxacin) produced significantly sized zones of inhibition of approximately 24 mm. However, the negative controls (dimethyl sulphoxide and distilled water) produced no zone of inhibitory. All the four extracts used showed good antibacterial activity with *Aloe secundiflora* showing the highest activity at low concentrations (MIC 5.5 mg/ml). *Bulbine frutescens* extract showed minimum activity (MIC 8.8 mg/ml).

The plant extracts were tested for the presence of secondary metabolites. Table 2 shows that all the methanolic leaf extracts from the plant had the four secondary metabolites being tested for.

The antimicrobial activity from the extracts against *Salmonella typhi* may be due to presence of saponins, alkaloids, tannins and flavonoid in the plant extracts; *Bulbine frutescens* [26]; *Vernonia lasiopos* [18]; *Aloe secundiflora* [20,27,28]; *Tagetes minuta* [29,30].

Plant extracts	MIC (mg/ml)	MBC (mg/ml)	Zone of Inhibition (mm)
<i>Aloe secundiflora</i>	5.5	7.3	16 ± 0.68
<i>Vernonia lasiopos</i>	5.6	7.5	13 ± 1.68
<i>Tagetes minuta</i>	6.1	8.2	17 ± 1.15
<i>Bulbine frutescens</i>	8.8	10.9	15 ± 0.15
+ve control	-	-	24
-ve control	-	-	-

**Key:** ± Standard error; **-ve control** (dimethyl sulphoxide and distilled water); **+ve control** (ciprofloxacin). **MIC:** Minimum Inhibitory Concentration; **MBC:** Maximum Inhibitory Concentration.

**Table 1:** Efficacy test of the plants leaf extracts against *Salmonella typhi*.

Plant extracts	Saponins	Tannins	Alkaloids	Flavonoids
<i>Aloe secundiflora</i>	+	+	+	+
<i>Vernonia lasiopos</i>	+	+	+	+
<i>Bulbine frutescens</i>	+	+	+	+
<i>Tagetes minuta</i>	+	+	+	+

**Key:** (+) present

**Table 2:** Phytochemical tests on the plant extracts.

## Conclusion

In conclusion, the present study will help us to use these medicinal plants as a source of herbal medicine to treat different infectious diseases caused by *Salmonella typhi* and other gram negative bacterial pathogens. The extracts can also be used as raw material in the manufacturing of conventionally used drugs against pathogenic bacteria that have developed resistance against standard antibiotics. Further isolation of the specific bioactive compounds responsible for the antimicrobial activity will give us other natural resources that can be used in treating bacterial infections.

## References

- Lichterhan BL (2004) Aspirin: The Story of a Wonder Drug. *British Medical Journal* 329: 1408.
- Fabricant DS, Farnsworth NR (2001) The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspective* 1: 69-75.
- Soule JA (1993) *Tagetes minuta* A Potential New Herb from South America: *New Crops*. Proceedings of the New Crops Conference, pp. 649-654.
- De las Rivas J (1989) Reversed-phase high performance liquid chromatographic separation of lutein and lutein fatty acid esters from marigold flower petal powder. *Journal of Chromatography* 464: 442-447.
- Basile A, Sorbo S, Giordano S, Ricciardi L, Ferrara S, et al. (1992) Antimicrobial resistance of *Shigella* isolates in Bangladesh, 1983-1990: increasing frequency of strains multiply resistant to ampicillin, trimethoprim-sulfamethoxazole, and nalidixic acid. *Journal of Clinical Infectious Diseases* 14: 1055-1060.
- Broussalis AM, Ferraro GE, Martino VS, Pinzon R, Coussio JD et al. (1999) Argentine plants as potential source of insecticidal compounds. *Journal of ethno pharmacology* 67: 219-223.
- Tereschuk ML, Riera MVQ, Castro GR, Abdala LR (1997) Antimicrobial activity of Flavonoids from leaves of *Tagetes minuta*. *Journal of Ethno pharmacology* 56: 227-232.
- Talmadge J, Chavez J, Jacobs L, Munger C, Chinnah T, et al. (2004) Fractionation of *Aloe vera* L. inner gel, purification and molecular profiling of activity. *International Immuno pharmacology journal* 4: 1757-1773.
- Habeeb F, Shakir E, Bradbury F, Cameron P, Taravati MR et al. (1994) Effect of garlic on lead contents in chicken tissues. *DTW* 101: 157-158.
- Ferro VA, Bradbury F, Cameron P, Shakir E, Rahman SR, et al. (2003) *In vitro* susceptibilities of *Shigella flexneri* and *Streptococcus pyogenes* to inner gel of *Aloe barbadensis* Miller. *Antimicrobial agents and chemotherapy* 43: 1137-1139.
- Kaingu F, Kibor A, Waihenya R, Shivairo R, Mungai L (2013) Efficacy of *Aloe secundiflora* Crude Extracts on *Ascaridia galli* *in vitro*. *Sustainable Agriculture* 2: 49-53.
- Kigundu EVM, Rukunga GM, Keriko JM, Tonui WK, Gathitwa JW, et al. (2009) Anti-parasitic activity and cytotoxicity of selected medicinal plants from Kenya. *Journal of ethno pharmacology* 123: 504-509.
- Acocck JPH (1975) Veld types of South Africa. *Memoirs of Botanical Survey of South Africa Botanical Research Institute, Pretoria*.
- Coopsamy RM, Magwa ML, Mayekiso B (2000) Proceedings: Science and Society University of Fort Hare, Bhishe, Eastern Cape, South Africa.
- Kelmanson JE, Jager AK, Van Staden J (2000) Zulu medicinal plants with antibacterial activity. *Journal of ethno pharmacology* 69: 241-246.
- Van Wyk BE (2008) A broad review of commercially important Southern African medicinal plants. *Journal of ethno pharmacology* 119: 342-355.
- Keeleya SC (2007) A phylogeny of the "evil tribe" (Vernonieae: Compositae) reveals Old/New World long distance dispersal: support from separate and combined congruent datasets. *Molecular Phylogenetics and Evolution* 44: 89-103.
- Kareru PG, Gachanja AN, Keriko J M, Kenji GM (2007) Antimicrobial activity of some medicinal plants used by herbalists in Eastern province, Kenya. *African Journal of Traditional Complementary And alternative Medicine* 5: 51-55.
- Kambizi L, Afolayan AJ (2001) An ethno botanical study of plants used for the treatment of sexually transmitted diseases (Njovhera) in Gुरुve District, Zimbabwe. *Journal of ethno pharmacology* 77: 5-9.
- Arunkumar S, Muthuselvam M (2009) Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. *World Journal of Agricultural Sciences* 5: 572-576.
- Joshua M, Ngonidzashe M, Bamusi S (2010) An evaluation of the antimicrobial activities of *Aloe barberdensis*, *A. chabaudii* and *A. arborescens* leaf extracts used in folk fore veterinary medicine in Zimbabwe. *Journal of animal and veterinary advances* 9: 2918-2923.
- Newall CA, Anderson LA, Phillipson JD (1996) *Herbal medicines*. The pharmaceutical Press, London.
- Eloff JN (1998) A sensitivity and quick microplate method to determine the minimal inhibition concentration of plant extracts for bacterial organisms. *Medicinal plants journal* 64: 711-713.
- Rabe T, Mullholland D, Van Staden J (2002) Isolation and identification of antibacterial compounds from *Vernonia colorata* leaves. *Journal of Ethnopharmacology* 80: 91-94.
- Parekh J, Nair R, Chanda S (2005) Preliminary screening of some folklore medicinal plants from Western India, for potential antimicrobial activity. *Indian journal of pharmacology* 37:406-409.
- Yakubu MT, Afolayan AJ (2009) Effect of aqueous extract of *Bulbine natalensis* (Baker) stem on the sexual behaviour of male rats. *International Journal of Andrology* 32: 629-636.
- Devaraj A, Karpagam T (2011) Evaluation of anti-inflammatory activity and analgesic effect of *Aloe vera* leaf extract in rats. *International Research Journal of Pharmacy* 2: 103-110.
- Mariappan V, Shanthi G (2012) Antimicrobial and phytochemical analysis of *Aloe vera*. L. *International Research Journal of Pharmacy* 3: 158-161.
- Irum S, Amjad H, Ummara WK, Mohammad MS (2010) Evaluating biological activities of the seed extracts from *Tagetes minuta* L. found in Northern Pakistan. *Journal of medicinal plants research* 4: 2108-2112.
- Lubna T, Naeem K (2012) Antibacterial potential of crude leaf, fruit and flower extracts of *Tagetes minuta* L. *Journal of Public Health and Biological Sciences* 1: 74-78.