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Anti-typhoid Activity of *Adhatoda vasica* and *Vitex negundo*

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Abstract: Typhoid activity is an acute systemic infection caused by *Salmonella typhi*.in present study the methanolic leafs extract of *Vitex negundo* and *Adhatoda vasica* were analyzed for anti-typhoid activity against *Salmonella typhi*. The leave sample was subjected to phytochemical analysis. The concentration of all the phytochemicals studied was higher in *Vitex negundo* than *Adhatoda vasica*, except alkaloids which was higher in *Adhatoda vasica* (11.3 ± 0.1mg/g) than *Vitex negundo* (8.6 ± 0.00 mg/g). Tannins were highest in both the plants, 61.3 ± 0.8 mg/g in *Adhatoda vasica* and 93.9 ± 0.8 mg/g in *Adhatoda vasica*. The antioxidant activity was determined and compared with BHA and reducing power was compared with Ascorbic acid. Both the samples had high antioxidant and reducing power activity. Several studies reported the loss of antioxidant system during infection of *Salmonella typhi*. The leaf extracts of *Adhatoda vasica* and *Vitex negundo* showed considerable antioxidant activity which can used as remedy against antioxidant system collapse and thus promises to be effective antioxidant supplement for typhoid patients. Besides antioxidant activity the leaves of both plants inhibited the growth of *Salmonella typhi.* The antibacterial activity of both leaf extracts were compared with gentamycin. The results of present shows that leaf extracts of *Vitex negundo* and *Adhatoda vasica* confer anti-typhoid activity against *Salmonella typhi*.

Key Words: Anti-typhoid, *Adhatoda vasica*, *Vitex negundo*.

Introduction

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols (Sies and Helmut, 1997). Antioxidants prevent the human system by neutralizing the free radicals interactively and synergistically. Plans are rich source of free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites which are rich in antioxidant activity (Aiyegoro *et al.,* 2010). The oxidation induced by reactive oxygen species (ROS) can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disorders. Although the body possesses such defense mechanisms, as enzymes and antioxidant nutrients, which arrest the damaging properties of ROS (Halliwell *et al*., 1995; Sies, 1993), continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them, and cause irreversible oxidative damage (Ferreira *et al*., 1993). Reactive oxygen species (ROS), include free radicals such as superoxide (O2-), hydroxyl radical (.OH), peroxyl radical (ROO.) as well as non-radical species such as hydrogen peroxide (H2O2) (Cerutti, 1991). In vivo, such species are securely coupled at their site of generation or are detoxified by endogenous antioxidative defences, so as to preserve optimal cellular function. In pathological conditions, however, the detoxifying mechanisms are often inadequate as excessive quantities of ROS are generated. This resulting pro-oxidant shift, a process known as oxidative stress can result in the degradation of cellular components viz., DNA, carbohydrates, polyunsaturated lipids and proteins or precipitate enzyme inactivation, irreversible cellular dysfunction and ultimately cell death, if the pro-oxidant-antioxidant balance is not restored. Furthermore, ROS play a cardinal role in the aetiology of numerous diseases (Halliwell and Gutteridge, 1989). In recent years, there is an increasing interest in finding antioxidant phytochemicals, because they can inhibit the propagation of free radical reactions, hence protect the human body from diseases (Kinsella *et al*., 1993). The pharmacological actions and the medicinal uses of extracts of leaves in folk medicine include the treatment of various types of infectious diseases. The importance of plant secondary metabolites in medicine, agriculture and industry has led to numerous studies on the synthesis, biosynthesis and biological activity of these substances. It has been estimated that over 40% of medicines have their origins in these active natural products (De Fatima *et al*., 2006, 2008; Vohra and Gupta, 2011). Polyphenols constitute a large group of naturally occurring substances in the plant kingdom, which include the flavonoids. The plant phenolics are commonly present in fruits, vegetables, leaves, nuts, seeds, barks, roots and in other plant parts. These substances have considerable interest in the field of food chemistry, pharmacy and medicine due to a wide range of favourable biological effects including antioxidant properties. The antioxidant properties of phenolics are mainly due to their redox properties. They act as reducing agents (free radical terminators), hydrogen donors, singlet

oxygen quenchers and metal chelators (Cook, 1996). Typhoid fever is global infection caused by the bacterium *Salmonella typhi*. The disease is transmitted by water, milk, fruits and vegetables contaminated with the bacterium. It is also transmitted by healthy carriers and contaminated food handlers. The bacilli may be carried mechanically from faeces to food by flies. Reptiles such as snakes, turtles, lizards and common domestic pets have been associated with transmission of *Salmonella typhi* (Birgitta *et al*., 2005). Typhoid fever can be treated with antibiotics; however resistance to common antimicrobial is widespread. In recent years there has been rapid rise in multi-drug resistance of *Salmonella typhi* against antibiotics all over the world.(Chin *et al*., 2002; Benoit *et al*., 2003; Abdullah *et al*., 2005).Several clinical treatment failures with fluoroquinolones (such as ciprofloxacin) in cases of *Salmonella typhi* has been reported in Europe, Asia and Africa (Butt *et al*., 2003; Nkemngu *et al*., 2005)In the present study, the methanolic and aqueous extracts of *Vitex negundo* and *Adhatoda vasica* have been screened for some phytochemical properties as well as antioxidant. Hence attributing to the above facts, the present study was up taken to study the phytochemicals, antioxidant and reducing power activity of *Vitex negundo* and *Adhatoda vasica*.

Material and Methods

Plant materials: The fresh tender leaves of *Vitex negundo* and *Adhatoda vasica* were collected from Ranchi (23° 21' 0" N LR, 85° 20' 0" E L). The leaves were washed with deionised water and disinfected with 0.1% $HgCl_2$ solution for 5min and dried in shade for 15 days. The dried materials were ground to fine powder with the help of electrical grinder (Jonani and Sondhi, 2002).*Vitex negundo* belongs to family verbenaceae is known as Five-leaf-Chaste tree. It is a large, aromatic shrub; with typical five foliate leave pattern found throughout the greater part of India at warmer zones and ascending to an altitude of 1500 m in outer western Himalayas. The shrub is one of the common plants used in Indian medicines. It has been claimed to possess many medicinal properties. The plant has been reported to have high medicinal value (Rajith and Ramachandran, 2010; Prajapati *et al*., 2004; Srivastava, 2009; Schütz *et al*., 2006) *Adhatoda vasica* belongs to family Acanthaceae known as Malabar Nut, is distributed throughout India up to an altitude of 1300m. The leaves, flowers, fruits, and roots are extensively used for treating cold, cough, whooping cough, chronic bronchitis and asthma as sedative, expectorant and antispasmodic in traditional medicines. It was also used by traditional midwives at the time of delivery. The plant has been reported to have high medicinal value (Rachna *et al*., 2011; Shanbhag and Khandagale, 2010).

Preliminary Phytochemical Screening: Qualitative phytochemical screening tests were conducted on the *Adhatoda vasica* and *Vitex negundo* leaf sample with previously published standards (Trease and Evans, 1989; Harborne, 1998)

Quantitative analysis of detected phytochemicals

Alkaloid determination: The alkaloid determination was done by following previously published work (Harborne, 1973). The sample was weighed in to a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added. Beaker was covered and allowed to stand for 4hr. then it was filtered and the extract was concentrated on water bath to one quarter of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to stand till settlement of precipitate. The precipitate was collected and washed with dilute ammonium hydroxide and filtered. Alkaloid was collected as residue and weighed after complete dryness and percentage was calculated and expressed in mg/g of plant extracts.

Tannin determination: The analyses of tannins content in fruits and vegetables were performed according to The International Pharmacopoeia (2003) and AOAC method (1965), after some modifications. 25 ml of the infusion were measured into 1 L conical flask, then 25 ml of indigo solution and 750 ml distilled deionised water (dd H_2O) were added. 0.1 N aqueous solution of $KMn0_4$ was used. The blue coloured solution changed to green colour. Standard solution of Indigo carmine was prepared as following: 6 g Indigo carmine was dissolved in 500 ml of distilled deionised water (dd H_2O) by heating, after cooling 50 ml of 95 - 97 % H2S04 was added, the solution was diluted to 1 L and then filtered. The blank tests by titration of a mixture of 25 ml Indigo carmine solution and 750 ml dd H_2O were carried out.

Calculations: The tannin content was calculated as percentage and expressed as mg/g of plant extract.

$$Tanin\,\% = \frac{(V - V_0) \times 0.004157 \times 250 \times 100}{g \times 25}$$

Where V in the volume of 0.1 N aqueous solution of $KMnO_4$ for titration of the sample (ml), V_0 is volume of 0.1 N aqueous solution of $KMnO_4$ for titration of the blank sample, ml; 0.004157 is tannins equivalent in 1 ml of 0.1 N aqueous solution of $KMnO_4$; g is mass of the sample taken for the analysis (g); 250 is the volume of volumetric flask (ml).

Saponin determination: Saponin was determined as per the published work of Obadoni and Ochuko (2001). 20 g of each grounded sample was put into conical flask and 100 cm^3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4hr. with constant stirring at about 55 ºC. The mixture was then filtered and the residue was again extracted with another 200ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90 ºC. The concentrate was transferred into a 250 ml separator funnel, added 20 ml diethyl-ether which as followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage and expressed as mg/g of plant extract.

Phenolic compounds determination: The amount of total phenol content, in various solvent extracts of flower was determined by Folin-Ciocalteu's reagent method(Karim *et al.*, 2011) .0.5ml of extract and 0.1 ml (0.5N) Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm. Gallic acid was used as a positive control. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of extracted compounds)

Flavonoids determination: Flavonoids were determined as per previously published work of Bohm and Kocipai – Abyazan (1994). 10 g of each sample was extracted with 100 ml of 80 % aqueous methanol repeatedly at room temperature. The whole solution was filtered through whatman filter paper #42 (125 nm). The filtrate was later transferred into crucible and evaporated into dryness over a water bath, the weight of the material and percentage quantity was calculated and expressed as mg/g of plant extract.

Extract preparation: 50 g of the sieved powder was weighed accurately and subjected to extraction in Soxhlet apparatus using ~350 ml methanol for preparation of methanolic extract. The obtained extract was concentrated with the help of vacuum rotatory evaporator at 45^oC and stored in air tight containers at room temperature and used for antioxidant analysis (Prieto *et al.*, 1999). Freshly

prepared aqueous leaf extracts were used for spectrophotometric analysis of reducing activity (Ferreira *et al*., 2007).

Anti-pathogenic assay: *Salmonella typhi* (MTCC 3216) was used during present experiment (procured from Hi-Media). The organism is a potential causative pathogen for typhoid fever.

Agar diffusion method: Following Threlfall *et al*. (1999), the agar plates were prepared and wells were made in the plate. Each plate was inoculated with 18 hr old cultures (100 µL, 10^4cfu) of the selected bacteria and spread evenly on plate. After 20 min, the wells were filled with different concentrations of samples. The control wells were filled with Gentamycin along with solvent. All the plates were incubated at 37 º C for 24 h and the diameter of inhibition zones were noted.

Broth dilution method: As proposed by walker (2000), the tubes containing the culture media were prepared, autoclaved and respective concentrations of the samples were added. Each tube was inoculated and respective concentrations of the samples were added. Each tube was inoculated with 18 h old cultures (100 µL, 10^4cfu). A control tube with inoculums and without any sample was prepared along with a sterile media tube as blank. All the tubes were incubated at 37 ºC on a shaker with 140 rpm for 24 h, the growth and hence the (minimum inhibitory concentration) MIC was measured at 660nm.

Results and Discussion

Phytochemical analysis: The results on phytochemical analysis of the leaf samples of *Adhatoda vasica* and *Vitex negundo* have been presented in Table – 1. The result reveals that tannin was highest in both the samples i.e. 61.3 ± 0.8 mg/g and 93.9 ± 0.8 mg/g in *Adhatoda vasica* and *Vitex negundo* respectively and phenols lowest among all the studied phytochemicals i.e. 1.3 ± 0.1 mg/g and 8.1 ± 0.1 mg/g in *Adhatoda vasica* and *Vitex negundo* respectively. The concentration of all the phytochemicals studied was higher in *Vitex negundo* than in *Adhatoda vasica* (Table – 1), except alkaloids which as higher in *Adhatoda vasica* (11.3 ± 0.1 mg/g) than in *Vitex negundo* (8.6 ± 0.00 mg/g). *Soladoye and Chukwana* (2012) reported tannin (4.98%), saponin (47.3%), alkaloid (2.49%), and flavonoids (6.48%) in *Cissus populnea.* Khan *et al.,* (2011) reported alkaloid content (1.13%) in *A. vasica,* 1.11% in*P. harmala*, 1.036% in *W. fruticosa*and 0.90%. in *V. cotinifolium* . 0.87% Phenolic content was observed in methanolic extract of *W. fruticosa*. Tannin content was recorded 15.75% in *M. rubicaulis,* 14.16%, *W. fruticosa,* 13.4% in *C. grata*, 12.33% in *V. cotinifolium*, 11.2% in *E. hirta*, 10.56% in *B. papyrifera* and 10.2% in *P. harmala*. Flavonoid content has been reported to be 10.95% in *V. negundo*. Saponin content was recorded 5.06% in methanolic extract of *T. officinale.*

Antioxidant activity: The results on antioxidant activity of the plants have been presented as table – 4. The results clearly reveals that the absorbance is directly proportional to the antioxidant activity (Fig -1; Fig – 2), i.e. more the absorbance, more is the antioxidant activity of the extract (Jayanthi and Lalitha, 2011) The total antioxidant activity of the *Vitex negundo* and *Adhatoda vasica* extracts expressed as the mg of ascorbic acid/100mg, which showed that both samples have good antioxidant capacity (Fig 1). Comparatively higher antioxidant activity was recorded for *Vitex negundo* which underlines its suitability as antioxidant supplement. Tiwari and Tripathi (2007) reported that non-polar fractions of *V. negundo* leaf trapped of free radicals and thereby inhibited lipid peroxidation which is reflected as antioxidant activity. Several reports emphasize that the type of solvents is also associated with antioxidant activities. Antioxidant activity of methanolic and hexane extracts of *Cordia wallichii* were examined and results showed the methanolic extract to be more effective (28.2%) than hexane extract

(16.7%). Sheikh *et al*., (2009) reported that antioxidant activity of some marine macro-algae depends on type of solvent used for extraction apart from other condition and non-polar solvents were more effective over aqueous solvents. Hence the methanolicsolvent was used for the present extraction. *Mshvildadze et al.* 2004 reported that antioxidant activities are directly related to the saponin content. *Rodriguez et al.* (2005) reported that the beneficial effects of saponin on serum lipids were related to a direct antioxidant activity of saponins. Elekofehinti *et al*., (2012) concluded that *Solanum anguivi* Saponins were capable of improving the antioxidant defense in rats. The antioxidant activity of Malasian *D. grandis* is mainly due to the saponin (18.9 mg/g) content of the plant. Kumar (2013) reported phenolic content in *Vitex trifolia (*74.5 GAE gm^{-1}), *T. chebula* (531.5 74.5 GAE gm^{-1}), *T. bellerica* (362.5 74.5 GAE gm^{-1}), *E. officinalis* (221.6 74.5 GAE gm^{-1}), *A. racemosus* (10.0 74.5 GAE gm^{-1}) and found a liner relation between antioxidant activity and phenolic contents of plants. Joabe Gomes de Melo *et al.,* (2010) screened some plants for their antioxidant activity and Tannin content. They reported highest tannin content in *Pyramidalis queiroz*(8.17 ± 0.64 µg/g), and lowest in *Cyperus distans* (1.22 ± 0.02 µg/g), they attributed the antioxidant activity of studied plants to their Tannin content. The antioxidant activity of *Vitex negundo* and *Adhatoda vasica* in present study seems to be due to their high content (Table -1) of tannins and Saponins. Flavonoid, phenols and alkaloids content (Table-1) are known to pose antioxidant properties to the plants (Hua, 2008; Nagai *et al*., 2003). Anti-dysenteric and anti-diarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids (Choudhury *et al*., 2012a, b). Various workers reported that there is a liner relation between the antioxidant activity and saponin, flavonoid, phenols, tannins and alkaloid contents (Cai *et al*., 2004; Tiwari and Tripathi, 2007). Akhmedov (1999) reported considerable decrease in the antioxidant system in chronic typhoid bacteria (*Salmonella typhi*) carriers; the use of antioxidants in combination with ampicillin normalized the antioxidant system. Bayin *et al*. (2012) reportedsignificant decrease ($p<0.05$) of oxidants among typhoid fever patients; antioxidants administration was effective in treatment of the fever. Thus *Vitex negundo* and *Adhatoda vasica* can be used as antioxidant supplements for treatment of typhoid fever.

Reducing power: The reducing ability of Ascorbic acid in comparison with *Vitex negundo* and *Adhatoda vasica* is shown in Table - 5. Both the plant leave samples were found to have high reducing ability. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay *et al.,* 2003). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary processes (Chanda and Dave, 2009*).* Tanic acid and Quercetin had highest reducing activity than ascorbic acid and Trolox. Norhaiza *et al.,* reported that there is a positive correlation between reducing capacities and individual antioxidative compounds in the order B-carotene > flavonoids > Vitamin C > Total anthocyanins > Phenolics.Darsini *et al.* deduced that the reducing capacity of banana increased with an increase in phenolic content (31.5 to 1000 mg/ml). Padma *et al.* (2013) concluded that the reducing power of methanolic extracts of *Imperata cylindrical* may be due to the presence of tannins (12.53 ± 0.56 mg/g) and phenolic (7.09 ± 0.14mg/g) compounds. Thus the reducing ability of the plants studied in this paper is attributed to the presence of Tannins, flavonoids and phenols.

Anti-pathogenic assay

Agar disc diffusion method: The results for antibacterial assay obtained by agar disc diffusion method are presented as Zone of inhibition in table – 2 and the agar plates are displayed as figure 1 to 3 showing ZOI of *Adhatoda vasica*, *Vitex negundo* and gentamycin respectively. The antibacterial property is measured in terms of zone of inhibition (mm); it is the area around the wells up to which the plant extract is able to inhibit the growth of bacteria. The more is the zone of inhibition the more is the antibacterial property of the tested plant sample. The zone of inhibition was calculated at different concentrations *viz.* 0.13 mg, 0.36 mg, 0.612 mg, 1.25 mg, 2.5 mg, 5 mg of both samples and was compared with zone of inhibition of standard antibiotic Gentamycin. Extract of both plant samples inhibited *Salmonella typhi*. The MIC of *Vitex negundo* and *Adhatoda vasica* against *Salmonella typhi* was recorded 0.612 and 2.50 mg/ml respectively. This shows that *Vitex negundo* is comparatively more effective against *Salmonella typhi* than *Adhatoda vasica*.

Broth dilution method: The MIC value obtained by broth dilution method is shown as table – 3. The MIC values showed that *Vitex negundo* (MIC – 5mg/ml) is more effective against *Salmonella typhi* than *Adhatoda vasica* (MIC – 9 mg/ml).

Several phytochemicals have been known to possess antibacterial properties. Tannins, alkaloids, saponins, flavonoids, and sterols have been found active against several pathogenic bacterial including *Salmonella typhi* (Kennedy and Wightman, 2011; Choudhury *et al*., 2013). Tannins have been known to form irreversible complexes with prolene rich protein resulting in inhibition of cell synthesis of bacteria (Mamtha *et al*., 2004). Flavonoids have been shown to inhibit several enzymes, chelate certain metal cations, affect protein phosphorylation (Middleton and Kandaswami, 1994) and interpose several membrane-linked processes (Smith, 1996) all of these phytochemicals are present in *Vitex negundo* and *Adhatoda vasica* , thus they seem to be effective against *Salmonella typhi* and can be used for suppression of typhoid fever. Many workers reported alkaloids to be effective against bacteria (Benbott *et al*., 2012; Maatalah *et al*., 2012; Karou *et al*., 2005). *Vitex negundo* and *Adhatoda vasica* contains high amount of antioxidant phytochemical (Kumar *et al*., 2013).

Table 1. Quantitative analysis of phytochemicals in *Vitex negundo* AND *Adhatoda vasica* (values in mg/g; n = 3)

Plantspecies	Alkaloids	Tannin	Phenols	Flavonoids	Saponin
Adhatoda Vasica	11.3 ± 0.1	61.3 ± 0.8	1.3 ± 0.1	2.1 ± 0.1	20.9 ± 1
Vitex negundo	8.6 ± 0	93.9 ± 0.8	8.1 ± 0.1	9.5 ± 0.1	30.3 ± 0.8

Table 2. Zone of inhibition (mm) and MIC of methanolic leaf extract of *Vitex negundo* and *Adhatoda vasica* against *Salmonella typhi* obtained by agar disk diffusion method

Concentrations (mg/ml)	ZONE OF INHIBITION (mm)	
	Vitex negundo	*Adhatoda vasica*
0.13	0	0
0.36	0	0
0.612	0.5	0
1.25	1.4	0
2.50	2.6	1.8
5.00	3	2.2
MIC (mg/ml)	**0.612**	**2.50**

Table 3. MIC of *Vitex negundo* and *Adhatoda vasica* obtained by broth dilution method against *Salmonella typhi*

Plant	*Vitex negundo*	*Adhatoda vasica*
MIC (mg/ml)	5	9

Table 4. Antioxidant activity expressed in µg/mg equivalent to ascorbic acid

Plants	Concentrations		
	5µg	50µg	100µg
Vitex negundo	**0.1 ± 0.0076**	**4 ± 0.0178**	**8 ± 0..43**
Adhatoda vasica	**0.2 ± 0.075**	**0.8 ± 0.0062**	**2.3 ± 0.08**
BHA	**11 ± 0.17**	**41 ± 0.055**	**65 ± 0.421**

Table 5. Reducing power of *Vitexnegundo* and Adhatoda vasica in comparison with ascorbic acid expressed in g/ml equivalent to ascorbic acid.

Plants	Different concentrations of plant samples		
	0.1g/ml	**0.5g/ml**	**1.0g/ml**
Vitex negundo	0.62 ± 0.004	0.67 ± 0.002	0.71 ± 0.015
Adhatoda vasica	0.65 ± 0.015	0.69 ± 0.025	0.73 ± 0.02
Ascorbic acid	0.77 ± 0.012	0.85 ± 0.013	0.9 ± 0.032

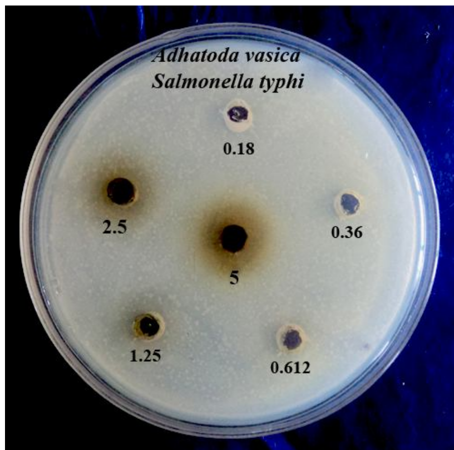

Figure 1. Zone of inhibition of *Adhatoda vasica* against *Salmonella typhi*

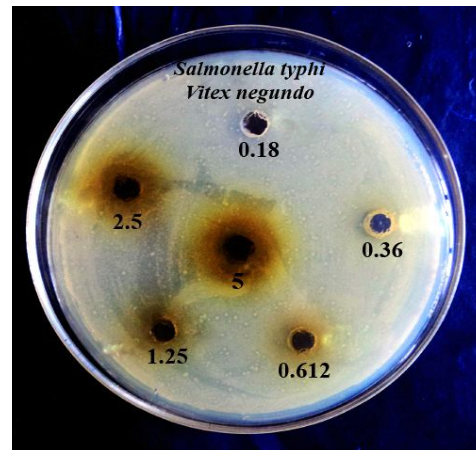

Figure 2. Zone of inhibition of *Vitex negundo* against *Salmonella typhi*

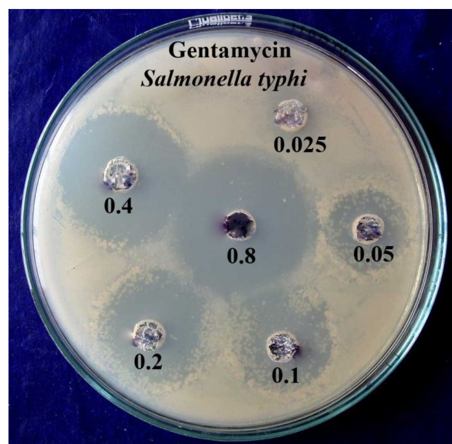

Figure 3. Zone of inhibition of Gentamycin against *Salmonella typhi*

Conclusions

The obtained results showed that methanolic extract was effective as an anti-typhoid agent against *Salmonella typhi.* The demonstration of anti-typhoid activity of *Adhatoda vasica* and *Vitex negundo* is indeed a promising development, which will help to discover new chemical classes of medicines (medicines) that could be used against the infection, which exhibits (multi drug resistance) MDR against synthetic medicines (antibiotics).

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