



***In vitro* antimicrobial activities of *Tithonia diversifolia* (Hemsl) A. gray extracts on two bacteria and fungus isolates**

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ABSTRACT

The *in vitro* antimicrobial activities of the aqueous and ethanolic extracts of *Tithonia diversifolia* leaf, stem and root were studied. Two bacterial isolates (*Escherichia coli* and *Salmonella typhi*) and fungus (*Candida albicans*) were subjected to susceptibility testing using two treatment levels of the extracts (0.05 and 0.1 g/ml). Solvents (aqueous and ethanol) and two antibiotics (30 µg Ampicillin and 5 µg Ciprofloxacin) were included as negative and positive control respectively. The solvents did not show appreciable growth inhibition when compared to the extracts and reference drugs. The ethanolic extract showed higher growth inhibitory effect on all the test organisms when compared to the aqueous extract except for *E. coli*. Similarly, the root and leaf extracts showed better growth inhibition compared to the stem. The inhibitory effect was found to be dose dependant. *E. coli* was more susceptible to the extracts as indicated by the mean zone of inhibition which decreased from 31.10 ± 1.01 mm (100%) in the positive control (Ampicillin) to 25.00 ± 0.50 mm (80%) in ethanolic root extract and 22.50 ± 0.58 mm (75%) in aqueous leaf extract at 0.1 g/ml concentration. Both ethanolic and aqueous extracts of the various plant parts were found to be less effective on *Salmonella typhi*. This is indicated by low zone of inhibition which ranged between 0.00 ± 0.00 mm (0%)-13.50 ± 0.29 mm (41%). The ethanolic and aqueous root extracts at 0.1 g/ml showed better potency on *Candida albicans* with mean inhibitory zone of 27.50 ± 0.87 mm (72%) and 25.00 ± 0.29 mm (79%) respectively. These values compared well with reference drug Ciprofloxacin. The results indicate that *T. diversifolia* leaf and root could serve as good sources of antimicrobial agent.

Keywords: Antimicrobial, inhibition, *Candida albicans*, *Escherichia coli*, *Salmonella typhi*, *Tithonia diversifolia*

INTRODUCTION

Mexican Sunflower (*T. diversifolia*) is a perennial broad-leaf weed species belonging to Asteraceae family. It originated in North and Central America and has become naturalised as invasive species in many tropical countries including Nigeria [7] [8]. In Nigeria, the weed has become predominant in waste lands, railway banks, building sites, fallowed land and cultivated farm lands [1].

As an aggressive weed species of arable crops, it is interesting to note that the plant has been found to be useful in the treatment of several ailments such as stomach pain, sore throat, indigestion, liver diseases and pains [11]. The leaf extract of this plant has been found to exhibit anti-malaria [5], anti-inflammatory [12], anti-proliferation [3], pesticidal [13], antifungal and antibacterial properties [4]. The local healers use the flower heads for treatment of wound and bruises [15]. The foregoing literature have established the abundance and varied use of this plant in the wake for search for the development of new chemotherapeutic agents. In this present investigation, the *in vitro* antimicrobial activities of *T. diversifolia* extracts (aqueous and ethanolic) on two bacteria and fungus isolates were evaluated. The overall intention is to provide information on which part of the plant shows appreciable growth inhibition on the test organisms.

EXPERIMENTAL SECTION

Collection of Bacterial and fungus isolates

Two gram negative bacterial pathogens, *Escherichia coli* and *Salmonella typhi* and a fungus, *Candida albicans*, used for the *in vitro* antimicrobial activities of the *T. diversifolia* parts were obtained from University College Hospital (UCH) Ibadan, Oyo State, Nigeria.

Collection and preparation of plant samples

Natural stands of *Tithonia diversifolia* growing at the University of Ilorin, Southern-Guinea savannah zone of Nigeria were carefully uprooted in November, 2013. The plant was authenticated by the curator as deposited by the first author at the University of Ilorin Herbarium (UIH) in the Department of Plant Biology with the Voucher number UIH 586. The plants were separated into leaves, stem and root and washed with distilled water to remove foreign particles. These were air dried under shade for 7 days after which the dried leaves, stem and root were ground into fine powder with the use of laboratory mill, sieved using 2.00 mm wire mesh and kept in separate plastic containers at room temperature (27-28°C) until they were subjected to aqueous and ethanolic extraction.

Extraction procedure

The aqueous extract of the dried powder was achieved by soaking 20 g of each of the plant parts (leaves, stem and root) in 200 ml of distilled water at room temperature (27-28°C) for 48hrs. The extracts were filtered through Whatmann filter paper No. 42 (125 mm) and concentrated by gentle evaporation on a heating mantle [4]. The ethanolic extract was done in similar manner as explained in the aqueous extract. The concentrated aqueous and ethanolic extracts were then preserved at 4°C in the refrigerator to prevent degradation until they were used for the *in vitro* antimicrobial assays at Kappa Biotechnology Laboratories, Ibadan, Nigeria.

Antimicrobial Sensitivity Testing Procedure

Sensitivity testing was done on each of the test pathogens for each of the extracts and antibiotics (Ampicillin and Ciprofloxacin) using porous paper disc of 4.75 mm in diameter [2]. Agar disc diffusion technique was used in the sensitivity testing using 0.05 and 0.1 g/ml concentration of the extract [9]. Sole solvents (aqueous and ethanol) and standard antibiotics (Ampicillin 30 µg and Ciprofloxacin 5 µg) were included as negative and positive controls respectively. The tests were conducted with the authenticated pure cultures of the test pathogens (*Escherichia coli*, *Salmonella typhi* and *Candida albicans*) to determine their respective tolerance to the extracts. To achieve this, sterile agar plates were aseptically inoculated with loopful of the test pathogens. Each inoculum was streaked evenly over the surface of the agar using a flamed inoculating loop by the spread plate technique. Using a flamed pair of forceps, the antimicrobial sensitivity discs prepared were embedded in 0.1g/ml of the extract, another in 0.05g/ml of the extract, the third in drops of the solvent (ethanol and sterile distilled water) and the fourth, a standard antimicrobial disc. These were carefully placed on the surface of the inoculated plates at a distance away from each disc to prevent over lapping. Thereafter, they were allowed to stand for 5 minutes to enable the extracts permeate into the medium, prior to incubation at 37°C for 24 hrs and 25°C for 120 hrs for bacteria and fungus respectively.

Minimum Inhibitory Concentration (MIC) determination

After incubation period, the diameter of inhibition halo around the extract-impregnated discs where test microorganism did not grow was measured for each disc. The extent of inhibition was determined by measuring the diameter of the inhibition zone using a transparent meter rule. The antimicrobial activities of the extracts of the plant parts were drawn from zone diameter of inhibition for each organism and compared with negative and positive controls.

RESULTS

The effects of different concentrations of aqueous and ethanolic extracts of *T. diversifolia* leaf, stem and root on *E. coli* are presented in Table 1. The zone diameter of inhibition regardless of solvents as well as the plant parts used for the extraction was in the range of 0.00 ± 0.00-27.00 ± 0.29mm (Table 1). At 0.1g/ml concentration of the extract, *E. coli* was sensitive to aqueous extracts (leaf and root) and ethanolic with zone diameter of inhibition of 22.50 ± 0.58mm, 27.00 ± 0.29mm and 25.00 ± 0.50mm respectively. The yield percent over the reference drug Ampicillin with zone diameter of inhibition of 31.1 ± 1.01mm (100%) was 72, 86 and 80 % respectively (Table 1). At 0.5g/ml, the potency of the extracts decreased as indicated by low zone diameter of inhibition which ranged from 0.00 ± 0.00-12.5 ± 0.29mm (Table 1). This showed that growth inhibition of the extract was concentration dependent. Among the plant parts, appreciable growth inhibition was not recorded under stem extract when compared to leaf and root (Table 1).

Table 2 shows that *Salmonella typhi* was less susceptible as shown by zone diameter of inhibition which ranged from 0.00 ± 0.00 - 13.5 ± 0.29 mm for all the extracts. These range values did not compare well with positive control drug Ampicillin with zone diameter of inhibition value of 33.0 ± 1.01 mm. The inhibitory effect of the extracts though less effective also increased with increase in concentration (Table 2).

As for the results in Table 3, it was observed that *Candida albicans* (fungus) was sensitive as indicated by zone diameter of inhibition which ranged from 0.00 ± 0.00 - 27.50 ± 0.87 mm for all the extracts (Table 1). At 0.1g/ml the ethanolic root extract and the aqueous root extract presented the highest activity zone diameter of inhibition of 27.50 ± 0.87 mm followed by the aqueous root extract (25.00 ± 0.29 mm) (Table 3). The yield percent of ethanolic root and aqueous root extracts over the reference drug Ciprofloxacin with zone diameter of inhibition values of 34.80 ± 0.46 mm (100%) was 72% and 79% respectively. Regardless of the plant parts, extracts at 0.05g/ml and negative control (solvents) were less effective against the fungus as indicated by zone diameter of inhibition which ranged from 0.00 ± 0.00 - 13.50 ± 0.29 mm (Table 3). Among the plant parts, the stem extract showed the lowest zone diameter of inhibition on the fungus compared to leaf and root extracts (Table 3). Generally, the ethanolic extract of the *T. divesifolia* showed better growth inhibition on all the test organisms than the aqueous extract with slight variation. Similarly, the susceptibility of the isolates was in decreasing order of *E. coli*, *Candida albicans* and *Salmonella typhi* (Tables 1, 2 and 3).

Table1: Inhibitory effects of different solvent extracts of *T. divesifolia* leaf, stem and root on *Escherichia coli*

Concentration	Zone of Inhibition (mm)					
	Aqueous			Ethanolic		
	Leaf	Stem	Root	Leaf	Stem	Root
0.05g/ml	11.00 ± 0.29 (35)	0.00 ± 0.00 (0)	12.50 ± 0.29 (40)	5.00 ± 0.29 (16)	5.50 ± 0.29 (18)	12.50 ± 0.44 (40)
0.1g/ml	22.50 ± 0.58 (72)	0.00 ± 0.00 (0)	27.00 ± 0.29 (86)	6.50 ± 0.50 (21)	7.00 ± 0.29 (23)	25.00 ± 0.50 (80)
Solvent control	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	5.00 ± 0.29 (16)	6.00 ± 0.29 (19)	6.50 ± 0.29 (21)
Ampicillin (AP 30 μ g)	----- 31.1 ± 1.01 (100)-----					

Values are expressed as mean \pm standard error of means

Values in parenthesis indicates percent growth inhibition over the reference drug

Table2: Inhibitory effects of different solvent extracts of *T. divesifolia* leaf, stem and root on *Salmonella typhi*

Concentration	Zone of Inhibition (mm)					
	Aqueous			Ethanolic		
	Leaf	Stem	Root	Leaf	Stem	Root
0.05g/ml	0.00 ± 0.10 (0)	3.50 ± 0.58 (11)	10.50 ± 0.29 (32)	9.50 ± 0.29 (29)	6.50 ± 0.29 (20)	5.50 ± 0.29 (17)
0.1g/ml	11.00 ± 0.29 (33)	4.00 ± 0.29 (12)	10.00 ± 0.76 (30)	10.00 ± 0.76 (30)	11.00 ± 0.29 (33)	13.50 ± 0.29 (41)
Solvent control	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	7.50 ± 0.29 (23)	5.00 ± 0.58 (15)	6.00 ± 0.58 (18)
Ampicillin (AP 30 μ g)	----- 33.0 ± 1.01 (100)-----					

Values are expressed as mean \pm standard error of means

Values in parenthesis indicates percent growth inhibition over the reference drug

Table 3: Inhibitory effects of different solvent extracts of *T. divesifolia* leaf, stem and root on *Candida albicans*

Concentration	Zone of Inhibition in mm and %					
	Aqueous			Ethanolic		
	Leaf	Stem	Root	Leaf	Stem	Root
0.05g/ml	8.00 ± 0.29 (23)	0.00 ± 0.00 (0)	12.50 ± 0.29 (36)	11.00 ± 0.29 (32)	9.50 ± 0.29 (27)	13.50 ± 0.29 (39)
0.1g/ml	10.50 ± 0.29 (30)	3.50 ± 0.29 (10)	25.00 ± 0.29 (72)	12.50 ± 0.29 (36)	12.00 ± 0.29 (34)	27.50 ± 0.87 (79)
Solvent control	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	11.50 ± 0.29 (33)	10.00 ± 0.50 (29)	12.50 ± 0.29 (36)
Ciprofloxacin (CIP 5 μ g)	----- 34.80 ± 0.46 (100)-----					

Values are expressed as mean \pm standard error of means

Values in parenthesis indicates percent growth inhibition over the reference drug

DISCUSSION

In this present investigation, the ethanolic extract of the plant generally exhibited broad spectrum activity against the test isolates compared to the aqueous extracts. Different solvents have been reported to have the capacity to extract

different phytoconstituents depending on their solubility or polarity in the solvent [6]. Ethanolic extracts in this study could have had higher solubility for more phytoconstituents, consequently the highest antimicrobial activity. The demonstration of antimicrobial activity by aqueous extracts provides the scientific basis for the use of these plants in traditional treatment of diseases, since most traditional medical practitioners use water as their solvent in which the decoctions are prepared [14].

The inhibitory effects of the extracts of the various parts were highly dependent on concentration. This coincides with observation made by Okigbo [9] in the potential inhibitory effects of ethanol and cold water extracts on the diameter of zone of inhibition (mm) at varying concentrations (mg/ml) of rhizome extracts of *Zingiber officinale* Rosc., *Curcuma longa* L. and *Discorea bulbifera* L. on some human pathogens (*S. aureus*, *E. coli* and *C. albicans*). The leaf and root extracts showed better potency against the test isolates most importantly on *E. coli* and *Candida albicans* than the stem extract. This could be attributed to high amount of active components in leaf and root than the stem. This present study further justifies the reports of Orwa [11] and Ogunfolakan [8] where *T. diversifolia* leaf was considered to have most of the active constituents than all other parts. As demonstrated in this study, the root of this plant could also be considered as having high amount of bio-active components with antimicrobial activity.

Earlier phytochemical screening carried out on *T. diversifolia* have shown that the plant contains an appreciable amount of secondary metabolites such as alkaloids, tannins, flavonoids, terpenoids, phenols and several other aromatic compounds that serve as defence mechanisms against foreign organisms [6] [10] [14]. The presence of these active substances could be used to explain the antimicrobial activity by the root and leaf extracts of *Tithonia diversifolia* which are more pronounced in these parts than the stem. Similarly Tona [16] showed that *T. diversifolia* contains sesquiterpenes lactones and Tagitinin with pesticidal activity, anti-amoebic activity and antibacterial effect. The susceptibility of some of the test isolates at high concentration of the extracts could be attributed to these compounds.

CONCLUSION

It may be inferred from this study that the leaf and root of *T. diversifolia* could serve as good sources of antimicrobial agent most importantly against *E. coli* and *Candida albicans* whose growth were greatly inhibited.

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