IMPACT OF EDAPHIC FACTORS ON ACTIVE COMPONENTS OF *TRIDAX PROCUMBENS* L. AND ITS EFFECT ON *ASPERGILLUS* SPECIES

Orotope, O. M. and *Umebese, C. E.

Department of Botany, University of Lagos, PMB 1029 Unilag Post Office, Akoka-Yaba, Lagos, Nigeria
*E-mail: cumebese@gmail.com
(Received: April, 2013; Accepted: July, 2013)

ABSTRACT

A study was conducted on the impact of edaphic factors on the phytochemicals of *Tridax procumbens* L. and their ability to inhibit the growth of three *Aspergillus* spp. (*A. fumigatus, A. flavus and A. niger*). Plants and their habitat soils were collected from five different locations: L1-L5 at the University of Lagos. Variations were observed in the soil pH, soil moisture content, soil profile and soil texture. Using the American soil texture standard triangle, L1-L5 had the following soil textures: silt-clay-loam (L1), silt-loam (L2), silt-loam (L3), clay-loam (L4) and silt-loam (L5). Though locations L2, L3 and L5 were classified as silt-loam, L5 soil had higher silt content and lower clay content (60 % and 10 % respectively). Location L5 soil was also slightly more acidic (pH 6.34) and drier (1.9% moisture content) than the other soils. Silt-loam locations produced *Tridax* plants with the highest concentrations of phytochemicals (0.240 mg g^{-1} carotene and 0.0607 mg g^{-1} flavonoids by L3 plants and 0.0897 mg g^{-1} tannins by L5 plants). All the aqueous extracts of plants from the five locations, were observed to inhibit the growth of the three *Aspergillus* spp that cause aspergillosis but L5 plant extract which had the highest tannin content, induced the highest growth inhibition. The zone of inhibition exhibited by the plant extract from location L5 was higher than that exhibited by antimycotic drug which served as positive control. This research reveals that the best soil type for growing *Tridax procumbens* L. for the highest concentrations of phytochemicals and the most effective antifungal activity is silt-loam with low clay and high silt contents, a pH of 6.34 and moisture content of 1.9 % in the dry season.

Keywords: *Tridax procumbens*, Edaphic factors, Phytochemicals, Antifungal, *Aspergillus* spp.

INTRODUCTION

Tridax procumbens L. is a flowering plant belonging to the Asteraceae family, tribe Heliantheae. Its common name includes coat buttons and tridax daisy in English (Bhagwat *et al*., 2008). It is a small perennial herb having short, 3-7 cm long hairy bladelike leaves that are simple, opposite, exstipulate and lanceolate to ovate. Flowers are tubular and of two types: ray florets and disc florets with basal placentation. They are yellow, hairy in capitulum inflorescence (Khan, 2008). Flowering and fruiting takes place throughout the year. The fruit is a hard achene covered with stiff hairs and has feathery, plume-like white pappus at one end (Jain and Jain, 2012).

The phytochemical screening of *Tridax procumbens* has revealed the presence of alkaloids, carotenoids, saponins, flavonoids (catechins and flavones) and tannins (Ikewuchi *et al.,* 2009). The proximate profile shows that the plant is rich in sodium, potassium and calcium. Verma and Gupta (2002) reported that the leaves of *Tridax* contain 26% crude proteins, 17% crude fiber, 39% soluble carbohydrates, 5% calcium oxide while the flowers contain luteolin, glucoluteolin, quercetin and isoquercetin. The plant also contains fumaric acid and fl-sitosterol.

Oleanolic acid has been obtained in good amounts from *Tridax* and found to be a potential antidiabetic agent when tested against -glucosidase (Muhammad *et al*., 2002). The aqueous and alcoholic extracts of leaves of *Tridax* cause marked decreases in the blood glucose level in the model of alloxan-induced diabetes in rats (Bhagwat *et al*., 2008). The aerial parts of *Tridax* have been shown to have hepatoprotective activity and significant protection in alleviation of D-Galactosamine/Lipopolysaccharide (D-GalN/LPS) induced hepatocellular injury (Vilwanathan *et al*., 2005). The extract of leaves of this plant also promotes wound healing in both normal and immune-compromised rats (Nia *et al*, 2003). It has antibacterial activity against four strains of bacteria: *Bacillus subtilis, Staphylococus aureus, Escherichia coli* and *Pseudomonas aeruginosa* (Mahato and Chaudhary, 2005).

Variations of secondary metabolites have been shown to occur in response to various types of stress (Watermann and Mole, 1989). Since some of these compounds may have negative impact on health when taken in high doses or may affect the quality of products, it is important to investigate the extent to which environmental factors alter phytochemicals and subsequent bioactivity of plants.

The objective of this study is therefore to investigate how edaphic factors of five different locations can alter the concentrations of phytochemicals in *Tridax procumbens* and the subsequent inhibitory effect on *Aspergillus fumigatus, A. flavus* and *A. niger*; the causal agents of aspergillosis. Many medicinal herbs especially some common weeds, such as, *Tridax*, are collected from the wild. A knowledge of the soil properties of the habitat from which this plant is collected may be a pointer to its degree of potency against aspergillosis..

MATERIALS AND METHODS

Plant Materials

Tridax procumbens L. (PBG0000010470) plants and the habitat soil samples were collected from five different locations (Highrise, L1; Faculty of Science, L2; Students Union Block, L3; Arts Block, L4 and Faculty of Education, L5) at the University of Lagos. Plants were identified in the Herbarium of the Department of Botany, University of Lagos.

Soil Analysis

Soil pH was recorded using digital pH meter and the percentage moisture content was determined. The soil profile of soils from the 5 locations was conducted and the standard soil textures were extrapolated from the U.S Texture Triangle (Saxton *et al*, 1986).

Preparation of Plant Extract

Plant samples collected from the 5 locations were cut into smaller sizes, air-dried at room temperature and ground into fine uniform powder using an electric milling machine. The aqueous extract of each sample was prepared by soaking 100 g of the dried powdered samples in 300 ml of distilled water for 96 hours. The extract was filtered and collected.

Phytochemical Screening

Tests for the active compounds: alkaloids, flavonoids, saponins, carotenes and tannins, in aqueous extracts were conducted using standard procedures described by Sofowora (1993), Trease and Evans (1983).

Quantitative Estimation of Chlorophyll A, Chlorophyll B and Carotene Contents

One gram of each powdered sample was homogenized with 5 ml of water. About 0.5 ml of the homogenate was taken and mixed with 4.5 ml of 80% acetone. They were all centrifuged and the absorbance was measured respectively, using spectrophotometer at 490, 645 and 663 nm wavelength using the following equation by Šesták (1971) and Lichtenthaler (1987).

Total Chlorophyll ($g\ l^{-1}$) = (0.0202) (O.D 645) + (0.00802) (O.D 663)

Chlorophyll A content ($g\ l^{-1}$) = (0.0127) (O.D 663) - (0.00269) (O.D 645)

Chlorophyll B content ($g\ l^{-1}$) = (0.0229) (O.D 645) (0.00488) (O.D 638)

Carotene content ($g\ l^{-1}$) = (O.D 490) (0.114) (O.D 663) (0.638) (O.D 645

Quantitative Estimation of Flavonoid

One gram of each extract was transferred into an Eppendorf tube containing 1 ml of distilled water and mixed with 75 µl of 5% of sodium nitrate. After 5 minutes 75 µl of 10% aluminum chloride solution was added. The mixture was allowed to stand for another 5 minutes and the 0.5 ml of 1M NaOH was added. Then mixtures were kept for 15 minutes. The absorbance was measured at 510 nm. Total flavonoid content was calculated using a standard Quecertin calibration curve and results were observed (Bao *et al*., 2005).

Quantitative Estimation of Total Amount of Tannins

One gram of each powdered sample was weighed into a 50 ml plastic bottle. 20 ml of distilled water was added and shaken for one hour in a mechanical shaker. They were filtered into 20ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1m HCL and 0.008M potassium ferrocyanide. The absorbance was measured at 720nm within 10

minutes. Also 5ml of tannin standard was pipetted into a test tube and mixed with 2ml of 0.1M FeCl3 in 0.1M HCL and 0.08M potassium ferrocyanide and the absorbance was measured at 720nm within 10 minutes (Van-Burden and Robinson, 1981).

Test Organisms

Three strains of *Aspergillus* spp (i.e. *Aspergillus fumigatus, A. flavus, A. niger*) were isolated and identified in the Department of Botany of the University of Lagos. Pure culture of the three strains were obtained and later used for inoculation.

Statistical Analysis

A single factor Analysis of Variance (ANOVA) was employed to determine the significant differences between observations (Zar, 1999).

RESULTS AND DISCUSSION

Tridax procumbens plants collected from five different locations (L1-L5) at the University of Lagos were found growing in soils having different profile, moisture content, pH and textures (Table 1). Using the American soil texture standard triangle (Fig.1, Saxton *et al.,* 1986), L1-L5 had the following soil textures: silt-clay-loam (L1), silt-loam (L2), silt-loam (L3), clay-loam (L4) and silt-loam (L5). Though locations L2, L3 and L5 had silt-loam, location L5 had higher silt content and lower clay content (60 % and 10 % respectively). Furthermore, location L5 soil was slightly more acidic (pH 6.34) and drier (1.9% moisture content) than the other soils. These variations in soil properties may have resulted in the observed differences in concentrations of phytochemicals (flavonoids, carotenoid and tannins; Figs.2, 3 and 4) in plants collected from the five habitat soils. Environmental factors, such as geographic location, growing season, soil type and mineral status are also known to influence levels of plant secondary metabolites (Lester and Eischen, 1996).

Table 1: Edaphic factors of five sample locations at the University of Lagos

Sample Locations	Moisture Content (%)	Soil pH	Soil Profile (%)			Soil Texture Standard*
			Silt	Clay	Sand	
L1	2.8	6.73	40	40	20	silt-clay-loam
L2	2.4	7.28	50	20	30	silt-loam
L3	2.25	6.76	50	20	30	silt-loam
L4	4.25	6.57	30	30	40	clay-loam
L5	1.9	6.34	60	10	30	silt-loam

*Soil texture extrapolated from Fig.1

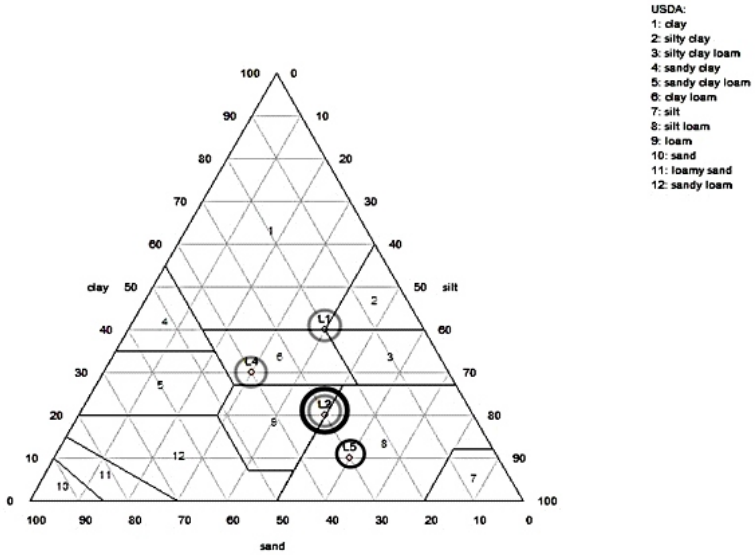

Fig 1: Soil triangle (American standard) indicating soil type found in L1, L2, L3, L4 and L5

Table 2: Phytochemical screening of leaf extract of *Tridax*

PHYTOCHEMICALS	STATUS
Alkaloids	+
Carotenes	++
Flavonoids	++
Saponins	+
Tannins	++

+ present, ++ present in high concentration

Tridax procumbens plants from L1-L5 were shown to have alkaloids and saponins in moderate quantites while flavonoids, carotenoids and tannins were in high concentrations (Table 2). According to Cowon (1999), flavonoids, carotenes and tannins are among several plant products utilized as antimicrobial agents; others being quinones, coumarines and terpenoids. Plants from the five locations had different concentrations of flavonoids, carotenes, tannins and chlorophylls (Figs. 2-5). The concentrations of carotenes and flavonoids were highest in location L3 (silt-loam) while location L5 (silt-loam) had the highest concentration of tannins. Location L2 produced plants with low concentration of carotene and tannins, though its silt-loam composition was similar to location L3. This may be partly due to the basic pH of location L2; the other two locations being slightly acidic. Thus, the differences in the phytochemicals of plants from these silt-loam locations may be attributed to the slight differences in pH, moisture content, percentage clay and silt contents.

Plant extracts from location L4 (clay-loam) exhibited the lowest concentrations of flavonoids and tannins. The presence of high clay content in this soil resulted in poor aeration compared to other soils; it was also waterlogged and polluted with detergents from car wash. These conditions make certain nutrients unavailable to plants causing diseases such as iron chlorosis (Singh *et al.,*2002). Soil characteristics have been shown to affect the levels of carotenoids in *Vitis vinifera* (Oliveira *et al.*, 2003). Lester and Eischen (1996) found that muskmelons grown on fine sandy-loam soils produced less -carotene than those on silt-clay-loam soils. In this study also, silt-clay-loam of location L1 was found to induce high carotene content in extracts of *Tridax*.

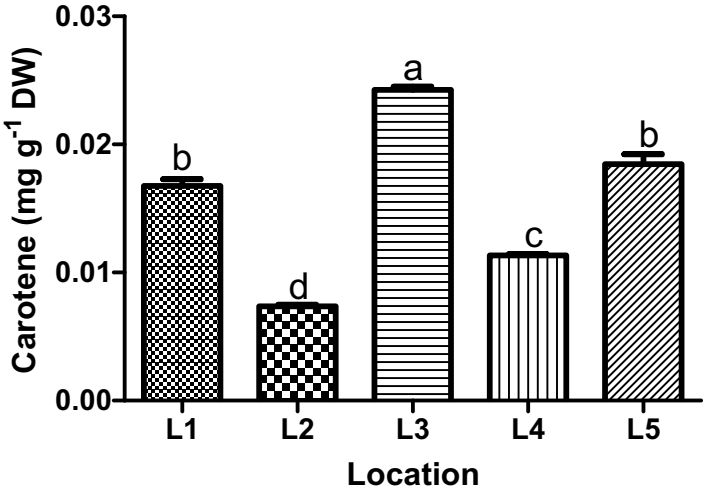

Fig. 2: Carotene Content of Leaf Extracts of *Tridax* Found in Different Locations (Bars with similar letters are not significantly different at $p<0.05$ using the Duncan's Multiple Range Test)

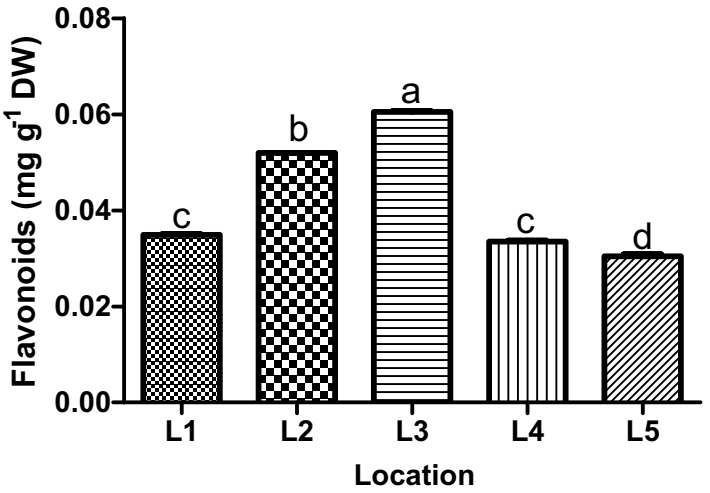

Fig. 3: Flavonoid Content of Leaf Extracts of *Tridax* Found in Different Locations (Bars with similar letters are not significantly different at $p<0.05$ using the Duncan's Multiple Range Test)

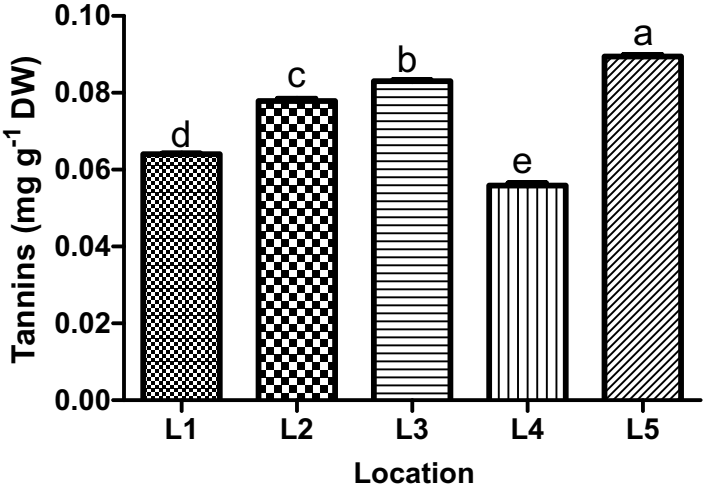

Fig. 4: Tannin Content of Leaf Extracts of *Tridax* Found in Different Locations (Bars with similar letters are not significantly different at $p<0.05$ using the Duncan's Multiple Range Test)

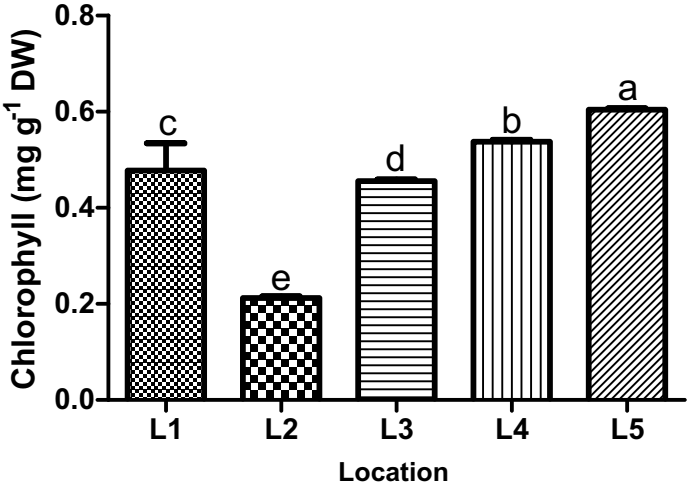

Fig. 5: Chlorophyll Content of Leaf Extracts of *Tridax* Found in Different Locations (Bars with similar letters are not significantly different at $p<0.05$ using the Duncan's Multiple Range Test)

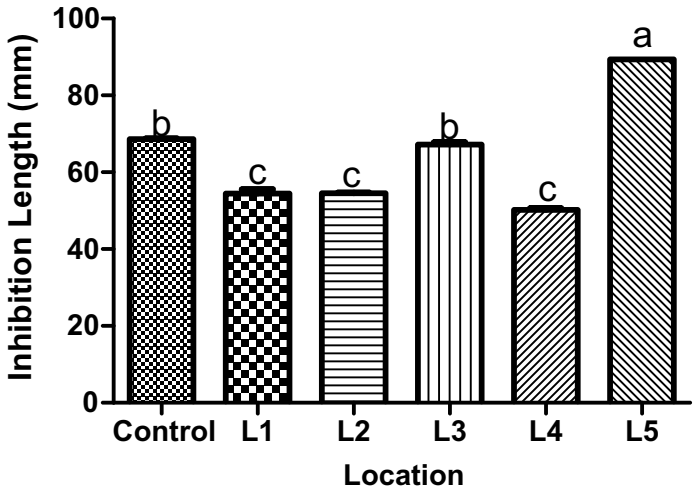

Fig. 6: Inhibition Lengths of *A. Fumigatus* Treated with Leaf Extracts of *Tridax* Found in Different Locations and Standard Control Drug (500mg Griseofulvin BP; Bars with similar letters are not significantly different at $p<0.05$ using the Duncan's Multiple Range Test)

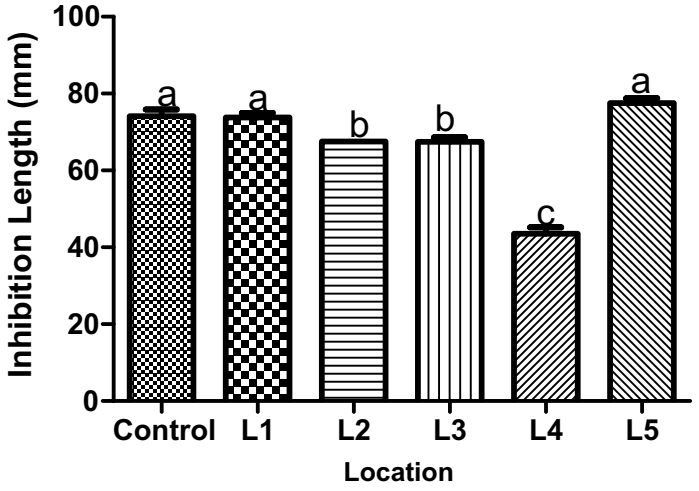

Fig. 7: Inhibition Lengths of *A. flavus* Treated with Leaf Extracts of *Tridax* Found in Different Locations and Standard Control Drug (500mg Griseofulvin BP; Bars with similar letters are not significantly different at $p<0.05$ using the Duncan's Multiple Range Test)

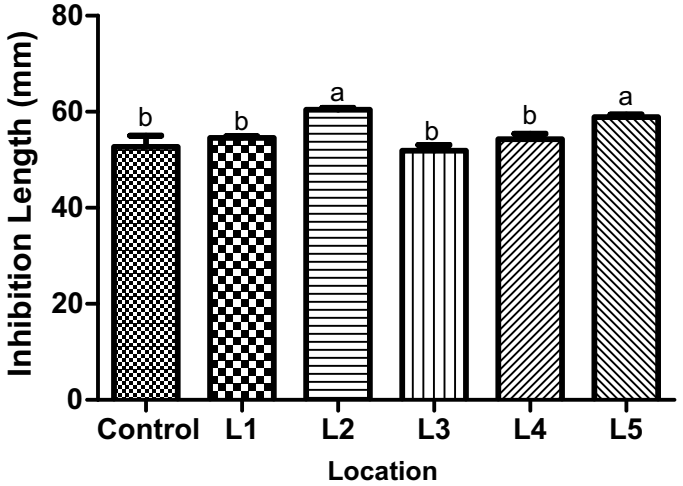

Fig. 8: Inhibition Lengths of *A. niger* Treated with Leaf Extracts of *Tridax* Found in Different Locations and Standard Control Drug (500mg Griseofulvin BP; Bars with similar letters are not significantly different at $p<0.05$ using the Duncan's Multiple Range Test)

Table 3: Inhibitory Lengths (mm) of *Aspergillus* Species Treated with Extracts of Leaves of *Tridax* from Different Locations

LOCATIONS	**Inhibition Lengths (mm)**		
	A. fumigatus	*A. flavus*	*A. niger*
L1	69±0.10b	74 ±1.00a	55 ±0.20c
L2	54±0.30c	74±0.70a	60±0.20b
L3	61±0.54b	68±0.30a	52 ±0.70b
L4	50 ±0.20b	44 ±1.00c	54 ±0.70a
L5	89 ±0.10a	78 ±0.70b	59 ± 0.30c
Griseofulvin (500 mg)	68.59±1.00b	74.13±3.00a	52.71±2.00c

Means of 12 replicates followed by different letters within the same row differ significantly at $p<0.05$

The aqueous extracts of leaves of *Tridax procumbens* showed inhibitory effect on three *Aspergillus* spp. (*A. fumigatus, A. flavus and A. niger,* Figs. 6, 7 and 8*)* that cause aspergillosis and other associated diseases. Extract from plants collected from location L5 exhibited highest concentration of tannins as well as the highest inhibitory effect on almost all the cultures of *Aspergillus* spp. (Figs. 6 and 7, Table 3). Futhermore, the inhibition of radial growth of the fungi treated with extracts of plants from L5 location was found to be higher than the control antifungal drug (500 mg Griseofulvin B.P). The least inhibition growth of the fungi was shown by plant extracts from location L4 (clay-loam) and this was caused by the presence of the lowest concentration of flavonoids and tannins which were reported to be antimicrobial agents (Cowon, 1999).

A comparison of the inhibitory effect of extracts from each of the five locations on the three *Aspergillus* spp. showed that extracts from location L5 exhibited the highest inhibitory effect on *A. flavus and A. fumigatus* (Table 3). Extracts of plants from locations L1, L2, L3, L5 and the control drug (Griseofulvin) caused lower inhibitory effect on *A. niger* compared with the other species, showing that *A. niger* is more resistant.

CONCLUSIONS

This study has revealed that *Tridax procumbens* L. plants growing in silt-loam locations (L2, L3 and L5) had the highest concentrations of flavonoids, carotenoids and tannins. Of the three locations, plants from L5 had the highest concentration of tannins and extracts of such plants had higher radial growth inhibition of *A. fumigatus, A. flavus* and *A. niger,* than soils from the other locations and the control drug, griseofulvin (500 mg). This suggests that peak concentration of tannins is induced in *Tridax* plants when they are grown in silt-loam soils which consist of 10 % clay, 30 % sand, 60 % silt, moisture content of 1.9 % and slightly acidic pH of 6.34. Thus, searching for increased potency of plants against diseases may involve using specific textures and properties of soil for cultivation. Further research includes the use of a wider range of soil textures to cultivate *Tridax* under controlled conditions.

REFERENCES

Bao, J. S., Cai, Y., Sun, M., Wang, G. Y. and Corke, H. 2005. Anthocyanins, flavonols, and free radical scavenging activity of Chinese bayberry (*Myricarubra*) extracts and their color properties and stability. *Journal of Agriculture and Food Chemistry* 53, 2327-2332.

Bhagwat, D.A., Killedar, S. G. and Adnaik, R. S. 2008. Antidiabetic activity of leaf extract of *Tridax procumbens*. *International Journal of Green Pharmacology* 2, 126 -128.

Cowan, M. M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews* 12, 564 - 582.

Ikewuchi, C. J., Ikewuchi, C. C and Igboh, M. N. 2009. Chemical profile of *Tridax procumbens* Linn. *Pakistan Journal of Nutrition* 8, 548-550.

Jain, A. and Jain, A. 2008. *Tridax procumbens* (L): A weed with immense medicinal importance: A Review. *International Journal*

of Pharma and Bio Sciences 3, 544-552.

Khan, S. K., Rahman, A. H. M. M, Alam, M. S., Ahmed F., Rafiul Islam, A. K. M. and Rahman M. M. 2008. Taxonomic Studies on the Family Asteraceae (Compositae) of the Rajshahi Division. *Research Journal of Agriculture and Biological Sciences* 4, 134-140.

Lester, G. E. and Eischen, F. 1996. Beta-carotene content of postharvest orange-fleshed muskmelon fruit: effect of cultivar, growing location and fruit size. *Plant Foods for Human Nutrition* 49, 191-197.

Lichtenthaler, H. K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol.* 148, 350 - 382.

Mahato R. B. and Chaudhary R. P. 2005. Ethnomedicinal study and antibacterial activities of selected plants of Palpa district, Nepal. *Scientific World* 3, 26-31.

Muhammad S. A., Muhammad J. S., Shazad, H. and Muhammad I.C. 2002. Inhibition of a-glucosidase by oleanolic acid and its synthetic derivatives. *Phytochemistry* 60, 295-299.

Nia, R., Paper, D. H., Essien E. E., Oladimeji, O. H. Iyadi, K.C. and Franz, G. 2003. Investigation into in-vitro radical scavaging and in-vivo anti-inflammatory potential of *Tridax procumbens. Nigerian Journal of Physiological Science* 18, 39-43.

Oliveira, C., Silva Ferreira, A. C., Mendes Pinto, M., Hogg,T., Alves, F. and Guedes De Pinho, P. 2003. Carotenoid Compounds in Grapes and Their Relationship to Plant Water Status. *J. Agric. Food Chem.* 51, 5967-5971.

Saxton, K. E., Rawls, W. J., Romberger, J. S. and Papendick. R. I. 1986. Estimating generalized soil-water characteristics from texture. *Soil Science Society of American Journal* 50, 1031-1036.

Šesták, Z. 1971. Determination of chlorophylls *a* and *b*. *In: Plant Photosynthetic Production: Manual of Methods* (Z. Šesták, J. Catsky, P.G. Jarvis (editors). Dr. W. Junk N. V. Publ., The Hague. pp. 672-701.

Singh, M., Müller, G. and Singh, I. B. 2002. Heavy metals in freshly deposited stream sediments of rivers associated with urbanization of the Ganga Plain, India. *Water Air and Soil Pollution* 141, 35-54.

Sofowora, A., 1993. *The State of Medicinal Plants in Nigeria.* 1st Edn., UI Press, Nigeria, pp: 206-210.

Trease, G. E. and W. C. Evans, 1983. *Textbook of Pharmacognosy*. 12th Edn., Tindall and Co., London, pp: 343-383.

Van-Burden, T. P. and Robinson, W. C. 1981. Formation of complexes between proteins and tannin acid. *J Agric. Food Chem.*1, 77.

Verma, R.K. and Gupta, M. M. 2002. Lipids constituents of *Tridax procumbens. Phytochemistry* 60, 295-299.

Vilwanathan, R., Shivashangari, K. S. and Devak, T. 2005. Hepatoprotective activity of *Tridax procumbens* against dgalactosamine/lipopolysaccharide-induced hepatitis in rats. *Journal of Ethnopharmacology* 101, 55-60.

Waterman, P. G and Mole, S. 1989. Extrinsic factors in influencing production of secondary metabolites in plants. In: Insect-Plant Interactions. E. A. Bernays (editor). Boca Raton, FL: CRC Press. Vol. 1 pp 107-134.

Zar, J. 1999. *Biostatistical Analysis.* 4th Edition. Prentice-Hall, U.S.A.