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Phytochemical and Antimicrobial Studies of Extracts from the Leaves of *Tithonia Diversifolia* for Pharmaceutical Importance

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Abstract:*Phytochemical screening of extracts from the leaves of Tithonia diversifolia displayed the presence of Alkaloids, Saponin, Saponin glycoside, Tannin, Balsam, Cardiac glycoside and Volatile oil. Spectrophotometric analysis for trace metals, Phosphorus and Sulphur showed that T. diversifolia contained Mn (0.490±0.001 mg/100g), Zn (1.609±0.001 mg/100g), Cu (0.454±0.001 mg/100g), Ni (0.758±0.001 mg/100g), Fe (0.690±0.002 mg/100g), P (55.62±0.200 mg/100g) and S (709±1.000 mg/100g). The medicinal properties of the extract were evaluated in-vitro by antimicrobial and antifungal assays. The aqueous extract (but not methanol and petroleum ether extracts) showed growth inhibitory effects on Staphylococcus aureus and Escherichia coli, but Pseudomonas aeruginosa and Saccharomyces cerevisiae were resistant to all the plant extracts and the antibiotic controls. The Minimum Inhibitory Concentration (MIC) of the aqueous extract of T. diversifolia on S. aureus and E. coli were both 12.50mg. The Minimum Bacterial Concentration (MBC) of the aqueous extract against the test organism ranged from 12.50mg to 25.00mg.*

Keywords: *antimicrobial, herbal, pharmaceutical, phytochemical, T. diversifolia*

I. Introduction

The use of plants and plant extracts for medicinal purposes has been going on for thousands of years; and it has been the source of much useful therapy in both herbalism and folk medicine (Bubayero, 1998). The Mexican Sunflower (*T. diversifolia*) belongs to the plant family known as compositae (*Asteraceae*). It is an annual broadleaf weed reported to have been introduced into West Africa as an ornamental plant (Akobundu and Agyakwa, 1997) but has become a weed problem in field crops, wasteland and roadsides (Baruah *et al*., 2000). The plant is about 2.5m high, bushy, much branched and perennial. It reproduces from seeds and through vegetative re-growth of the basal stem when the plant is cut. The stem is quadrangular, spirally-ridged, pubescent below and glabrous above. The leaves are simple, alternate, lobed and of about 5-15cm long and 3.5-6cm broad. It is dark green, toothed and wedged shaped at the base (Akobundu and Agyakwa, 1997). The inflorescence is a solitary capitulum on a peduncle 7-15cm long with large orange-yellow florets, while the fruit is compressed and about 6mm long.

Not much has been reported about *T. diversifolia* in Nigeria, because it is a relatively new introduction to the Nigerian weed ecology (Bubayero, 1998). Preliminary observation by Akobundu and Agyakwa, (1997) showed that this shrub is an aggressive weed because it grows rapidly, forming large communities and eliminates other plants by forming canopy over them, thus cutting off light supply. However, some research findings reported the activities of *T. diversifolia*. Tongma *et al.* (2008) reported the allelopathic activity of the Mexican sunflower in the soil. Aqueous extract of the leaves of this plant applied to soil was shown to inhibit the growth of test plant species. Thus, the extract of the leaves was confirmed to be phytotoxic, and contain some allelochemicals. Baruah *et al*. (2000) were able to isolate growth inhibitory sesquiterpene lactones and a flavone from *T. diversifolia*. It is interesting to observe that a weed like this plant could also contain a natural pesticide, owing to its pesticidal activity. Schuster *et al*. (1999) reported the isolation of sesquiterpene lactones from two *Tithonia* species; *Tithonia diversifolia* and *Tithonia rotundifolia*. The sesquiterpene lactones isolated were found to be pesticidal on test plants. Thus, the aim of this study is to investigate the various phytochemical and anti-microbial properties of the leaves of *T. diversifolia* that are available for medicinal, pharmaceutical and agricultural use.

II. Materials And Methods

The leaves of *T. diversifolia* were collected along Federal University of Technology, Akure expressway, in Ondo State and along Asa Dam express bye-pass, Ilorin, Kwara State and in Ogbomosho in Oyo State. The plant species was later identified and authenticated by the department of Botany, University of Ilorin, Kwara State. The leaves were dried at $32^{o} \pm 2^{o}C$ for two weeks on a clean pavement prior the analysis. The drying process was further enhanced by the harmattan wind.

Sampling

The dried bulk samples of the leaves were pulverized using pestle and mortal, and sieved through a $2mm^2$ wire mesh to obtain a fine powder. The powdered samples were mixed together and quartered to obtain a representative sample weighing 150g.

Aqueous Extracts

20g of powdered leaves of *T. diversifolia* was weighed into 250ml beaker and 150ml of distilled water was poured unto the beaker content. The solution was stirred with a glass rod and allowed to soak for 24 h. The aqueous extract was filtered thrice through a plug of absorbent cotton-wool in a glass funnel. The aqueous extract was then filtered through 11cm Rundfilter paper MN713. The solution was concentrated by gentle evaporation on a heating mantle and poured into a 100ml beaker.

Methanolic Extracts

200ml of methanol was measured into the round-bottom flask of the soxhlet. 20g of the powdered leaves was placed in the sample container (i.e. thimble) of the soxhlet. The apparatus was coupled and the system was switched on at thermostat temperature of 65^oC. The sample was continuously extracted under reflux for 3 h, and the extract was poured into 100ml flask. Methanolic extract of the sample was concentrated by gentle evaporation on a heating mantle.

Petroleum Ether Extracts

200ml of petroleum ether was measured into the round-bottom flask of the soxhlet. 20g of the powdered leaves of *T. diversifolia* was placed in the thimble of the soxhlet. The apparatus was coupled and the system was switched on at thermostat temperature of 60^oC. The sample was continuously extracted under reflux for 3 h and the extract was poured into 100ml beaker after some of the petroleum ether had been recovered. The 100ml extract of the sample was concentrated by gentle evaporation on a heating mantle.

Phytochemical Screening of Crude Extracts

Phytochemical screening of the crude extract for saponin, saponin glycoside, tannin, anthracene, alkaloid, volatile oil, balsam and cardiac glycoside were carried out by the methods described by Evans (1999), Harbone (1993) and Sofowora (1999).

Spectroscopic Analysis of Crude Extracts

Methods of Howtz (1990), Skoog *et al*. (2006) and Pavial *et al*. (1992) were used for spectroscopic analysis of the samples, using Atomic Absorption Spectrophotometer (A200).
Colorimetric determination of Phosphorus was done using Vanadomolybdate (Yellow) method (AOAC, 2000). Spectrophotometric determination of Sulphate was done using Turbidometric method (AOAC, 2000). Antimicrobial assay of crude extracts of *T. diversifolia* was done using the methods described by Egwari (1999), Ntiejumokwu and Kolawole (1990), and WHO (1991) to test the effects of crude extracts on the following pathogenic microorganisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Saccharomyces cerevisiae*. Determination of antibiotic activity and antibiotic control was done by using the Disc Diffusion and Agar Diffusion techniques as described by WHO (1991). Determination of Minimum Inhibitory Concentration (MIC) of the crude extracts was done by using Tube Dilution method as described by Rotimi *et al*. (1999).

III. Results

Table 1 gives the phytochemical compounds present in crude extract of the leaves of *T. diversifolia*. The extracts were positive for some of the following compounds; alkaloids, anthracene, balsam, cardiac glycoside, saponin, saponin glycoside, tannin and volatile oil indicating their presence in the extract.

Table 1: Phytochemical Compounds in leaves of *T. diversifolia*

Phytochemical Compounds	Remarks
Alkaloids	+ve
Anthracene	-ve
Balsam	+ve
Cardiac glycoside	-ve
Saponin	+ve
Saponin glycoside	+ve
Tannin	++ve
Volatile oil	+ve

Key: ++ve = strongly positive, +ve = positive, -ve = negative

Table 2 shows the trace metal contents of the plant extract in mg/100g. The plant samples contained Manganese, Zinc, Copper, Nickel and Iron, while Table 3 shows the concentration of Phosphorus and Sulphur content of the extract in mg/100g.

Table 2: Trace Metals content in mg/100g

Elements	Conc. (mg/100g)
Manganese	0.490±0.001
Zinc	1.609±0.001
Copper	0.454±0.001
Cobalt	ND
Cadmium	ND
Nickel	0.758±0.001
Iron	1.690±0.002
Lead	0.031±0.002

The value represents mean ± SD (N=3), ND = Not Detectable

Table 3: Phosphorus and Sulphur concentration of the extract

Elements	Conc. (mg/100g)
Phosphorous	55.62±0.200
Sulphur	709.00±1.000

The value represents mean + SD (N=3)

Table 4 gives the inhibitory effects of extract of *T. diversifolia* leaves at 15mg. *Staphylococcus aureus* and *Escherichia coli* were sensitive to aqueous extract of *T. diversifolia* with zone diameters of inhibition of 6mm and 10mm respectively. However, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* (a fungus) were resistant (i.e. shows no growth inhibition) to extract of *T. diversifolia* and the antibiotic controls. *Staphylococcus aureus* and *Escherichia coli* were sensitive to the antibacterial effects of Tetracycline hydrochloride (15mg) and Ampicillin trihydrate (15mg) which were used as positive controls, with zone diameter of inhibition of 22mm and 26mm respectively (for ampicillin) and 26mm and 27mm respectively (for tetracycline). Table 5 and 6 show the Minimum Inhibitory Concentrations (MIC) of the extracts on pathogens; the MIC of extracts of *T. diversifolia* on both *Staphylococcus aureus* and *Escherichia coli* was 12.50mg.

Table 4: Inhibitory Effects of Extracts of Leaves of *T. diversifolia* (15mg)

	Zone diameter (mm) of growth inhibition				
Pathogens	**Aqueous**	**Methanol**	**Pet. Ether**	**Ampicillin Control**	**Tetracycline Control**
Staphylococcus aureus	6	0	0	22	26
Pseudomonas aeruginosa	0	0	0	0	0
Escherichia coli	10	0	0	26	27
Saccharomyces cerevisiae	0	0	0	0	0

Table 5: The MIC of *T. diversifolia* on *Staphylococcus aureus*

Extract	Concentration (mg/100g)	Growth Indication	MIC (mg/100g)
A1	25.00	Nil	
A2	12.50	Nil	12.50
A3	6.25	+	
A4	3.13	+	
A5	1.56	+	

Key: A = Aqueous extract
+ = Positive growth
Nil = No growth

Table 6: The MIC of *T. diversifolia* on *Esherichia coli*

Extract	Concentration (mg/100g)	Growth Indication	MIC (mg/100g
A1	25.00	Nil	
A2	12.50	Nil	12.50
A3	6.25	+	
A4	3.13	+	
A5	1.56	+	

Key: A = Aqueous extract
+ = Positive growth
Nil = No growth

IV. Discussion

The study showed (Table 1) that the leaves of *T. diversifolia* contained Saponin, Alkaloids, Saponin glycoside, Tannin and Balsam. This result agrees with similar research done by Kela *et al*. (1999); Menut *et al*. (2002) and Okogun (1996). These phytochemical compounds have pharmacological effects and have been the basis of chemical synthesis of drugs used in modern medicine responsible for their medicinal use in traditional medicine (Sofowora, 2001) and (Okogun, 1996). Saponins are found in most plants as nitrogen-free glycosides, each consisting of a sapogenin and a sugar molecule. Glycosides are large and varied groups of naturally occurring plant products, characterized, on hydrolysis, by the formation of sugar and non-sugar moiety. Schuster *et al*. (1999) and Egwari (1999) have isolated steroidal glycosides such as Hecogenin, Progesterone, Testerone and Diosgenin from plants and are now being used therapeutically as hormones and contraceptives in medicine. Cardiac glycosides, digitoxin and digoxigenin have varying effects in the cardiovascular systems of human. They are used in the treatment of heart disorders and high blood pressure (Groth, 1994 and Stenlake, 1997). Tannins are polyphenolic compounds also used for medicinal purposes e.g. catechol, hydroquinine and resorcinol are phenolic salicylates used as analgesics, antipyretics and as internal antiseptics in medicine and surgery (Bello, 1999 and Stenlake, 1997). Tona *et al.* (2008) showed that *T. diversifolia* contain sesquiterpenes lactones and Tagitinin with pesticidal activity, anti-amoebic activity and antibacterial effect. Therefore, the antimicrobial effect of crude extract of *T. diversifolia* on the growth of pure isolates of *Staphylococcus aureus* and *Escherichia coli* may be attributed to these compounds. Thus, the presence of these phytochemicals in *T. diversifoila* could be responsible for the observed pharmacological effects and their medicinal use in traditional medicine. Analysis of trace metals (Table 2) showed that *T. diversifolia* contains Mn, Zn, Cu, Ni, Fe and Pb. The concentrations (mg/100g) of Fe and Zn (Table 3) are very high, which suggests the presence of some bio-organic compounds. In the contrast the concentration of Pb is the lowest and therefore in the permissible level of consumption of the leave decoction. The high concentration of sulphur could be responsible for the antimicrobial (medicinal) properties, since sulphur-containing compounds are known for their antibiotic effects (Stenlake, 1997). For instance, the clinical effectiveness of sulphanimides in the control of bacterial infection has led to their use against pneumonia and streptococci infections (Tona *et al.*, 2008). Groth (1994) and Stenlake (1997) remarked that mere isolation and structural elucidation f plant extract may not be too significant, until appropriate bioassays are carried out to establish the biological activity exhibited by plant extracts. Therefore, microbiological screening of the crude extracts of *T. diversifolia* was carried out to establish its antimicrobial effects on pure isolates of pathogenic bacteria and fungi *consisting of S. aureus, P. aeruginosa, E. coli* and *S. cerevisiae*. The study showed that aqueous extracts of *T. diversifolia* possesses antimicrobial effect against the growth of pure isolate of *S. aureus and E. coli*. These findings are similar to that reported by Egwari (1999) and Rotimi *et al*. (1999). The result of zone diameters of inhibition of the plant extract on the growth of *Staphylococcus aureus* and *Escherichia coli* (Table 4) compared favourably with that of standard antibiotic controls consisting of *Tetracycline hydrochloride* (15mg) and *Ampicillin trihydrate* (15mg) (WHO, 1991 and Cheesebrough, 2000). *Pseudomonas aeruginosa* was resistant to all the plant extract and antibiotic controls. This observation agrees with that of Timothy and Nelson (1992) and Cheesebrough (2000). The plant extract had no antifungal activity against *Saccharomyces cerevisiae*. The Minimum Inhibitory Concentration (MIC), which is the concentration that permitted no visible growth after 24 h of incubator; was found to range from 1.56mg to 6.25mg for all the plant extracts (Table 5 & 6). The Minimum Bactericidal Concentration (MBC) of the plant extracts against the test organisms ranged from 12.50mg to 25.00mg. Therefore, the industrial utilization of the extracts of *T. diversifolia* as raw material for pharmaceutical industry has high prospect because of its antibacterial activity, availability, solubility and percentage yield.

V. Conclusion

The phytochemical screening of the crude extract of *T. diversifolia* tested positive for the presence of Saponin, Alkaloids, Saponin glycoside, Tannin and Balsam. The concentration (mean$\pm$SD) of elements analysed in mg/100g exhibited S > P > Fe > Zn > Ni > Mn > Cu > Pb. The high concentrations of sulphur and phosphorus

signified an index for the plant's medicinal properties. The medicinal properties of the plant as evaluated *in-vitro* by antimicrobial assay revealed that aqueous extract showed growth inhibitory effects on *Staphylococcus aureus* and *Escherichia coli*. However, *Pseudomonas aeruginosa* was resistant to the plant extract and antibiotic controls. The plant extracts have no antifungal effects on *Saccharomyces cerevisiae*.

VI. Recommendations

Further work is recommended on isolation and characterization of active chemical compounds responsible for the antimicrobial/antibacterial properties of the plant. Medicinal plants are known to exhibit seasonal variation in chemical properties and bioactivity, which could also affect their medicinal properties at any given period of time. Therefore, there should be an investigation to mitigate the seasonal variation in chemical properties of this plant.

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