Antimicrobial activity of leaf and stem extracts of *Andrographis echioides* (L.) Nees.

FRANCIS XAVIER T[1] AND SATHISH KUMAR D[1]*,
[1]Assistant professor, Department of Botany, St, Joseph's College Tiruchirappalli - 620 002, Tamil Nadu, India
[1]Research scholar, Department of Botany, St, Joseph's College Tiruchirappalli - 620 002, Tamil Nadu, India

SATHISH KUMAR D[1]*
Research scholar, Department of Botany, St. Joseph's College Tiruchirappalli-620 002, Tamilnadu, India.

Abstract

An antimicrobial efficiency of leaves and stem parts of *Andrographis echioides* (L.) nees were studied by using seven different solvent extracts tested against four human pathogenic bacteria such as *Staphylococcus aureus, Bacillus cereus, Serratia marcescens* and *Klebsiella pneumoniea*. Fungal pathogens like *Candida albicans* and *Aspergillus niger.* Both leaves and stem extract exhibited significant activity against selected human pathogens. The ethanol, methanol, acetone, ethyl acetate extracts showed highest zone of inhibition against the entire tested microorganism. The lowest activity observed on chloroform extract. No zone of inhibition determined on petroleum ether and aqueous plant extracts except *Staphylococcus aureus* lowest zone was founded. Completely no activity found in all the solvent extracts against *Aspergillus niger*. The results approve that *A. echioides* can be used as a source of medicine to fight infection affected by susceptible bacteria and fungi.

Key words - Antimicrobial activity, Disc diffusion assay, Human pathogens and Medicinal plant

Introduction

Contrary to the synthetic drugs, antibacterial activities of plant origin are associated with lesser side effects and have an enormous therapeutic potential to heal many infectious diseases. The potential for developing antibacterial from greater plants appears rewarding as it will lead to the development of a phytomedicine to act against microorganism. Currently a number of clinically effective antibiotics are becoming less active due to development of resistance. So, biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens (Kumaraswamy *et. al*., 2008).

There is unparalleled demand for natural remedies, green health products, pharmaceuticals, food enhancements, cosmetics, and herbal pesticides, which is bringing about this alarming loss of plant biodiversity. It is valued that 70-80% of people worldwide rely mainly on traditional, largely herbal remedy to meet their primary healthcare desires (Farnsworth and Soejarto, 1991; Shengji, 2001). Traditionally, mankind has been reliant on plants for food, flavors, homeopathic and many extra uses. Ancient written records of many developments (i.e. Egyptian, Roman and Chinese) give strong evidence regarding use of medicinal plant (Cowan, 1999), for example ayurveda booklets highest the use of medicinal plants to cure many diseases (Micke *et al*, 2009: Patwardhan *et al*, 2005). Considerable attention has been paid to use environmental-friendly and bio-friendly plant- based products for the prevention and cure of different human diseases. Since the adverse effects of synthetic drugs, the western population is looking for natural medicines. (Gijtenbeek *et al.,* 1999)

Acanthaceae family is a large family of dicotyledonous herbs, shrubs, or twining vines and some are epiphytes only flowering plants. Involve of 4300 species in 346 genera placing it among the top 12 most diverse families of flowering plants in all over the world. The four main centers of distribution are tropical,

subtropical and temperate regions of Indonesia and Malaysia, Africa, Brazil and Central America while also found in of Asia (Wasshausen and Wood, 2004). *Andrographis echioides* is belongs to Acanthaceae family and this species an herb widely spread in the dry districts of tropical India and Srilanka (Gambal, 1956). However, information on the chemical composition and bioactivity of this species is very rare (Shen *et al*., 2013). The whole plant extract is applied topically over fungal infections, to control hair fall, snake bite, cuts and wounds (Anonymous, 1990). Leaf extract heated with coconut oil used to control falling and gray of hair (Pandi Kumar *et al*, 2007). The plant from genus Andrographis is used in goiter, liver ailments (Nadkarni *et al*., 1976), fertility problems, bacterial (Qadre *et al*, 2009), malarial and fungal illnesses (Pandi kumar *et al*., 2005). Anti-inflammatory activity of this plant was thoroughly considered by (Basu *et al*, 2009). Freshly, *in vitro* antioxidant potential of *A. echioides* was reported (Ruba and Mohan, 2016).

Materials and Methods

Collection of Plant Material

The fresh leaves and stem of *Andrographis echioides* plant was used for this investigate. It was collected from Turaiyur region, Trichy district in Tamil Nadu.

Preparation of Plant Powder

The selected healthy leaves and stem parts were spread out and shade dried in the laboratory at room temperature for 5-8 days or until they broke easily by hand. The dried plant parts were crushed to a fine powder by using an electronic blender and the powders were kept in a closed container at room temperature for further usages. Shade dried plant material could be used as a source for secondary plant components. Most scientists have recommended using dry material for several reasons, traditional healers frequently used dried plant materials; the time delay between collecting plant materials and processing, it is time consuming and there are fewer problems associated with the large scale extraction of dried plant materials. Dried materials are more commonly used since old-dried materials may undergo some quantitative losses and qualitative changes. In the present investigation also shade dried plant materials were used.

Solvent Extracts

Fifty grams of the powdered plant materials were soaked respectively with 300 ml of each of the solvents *viz.*, ethanol, methanol, acetone, ethyl acetate, chloroform, petroleum ether and aqueous at the end of 48hrs each extract was filtered through Whatman No.1 filter paper and filtrates were focused. The filtered extracts were stored with bottles and it was kept in room temperature.

Antimicrobial Activity (Disc diffusion method)

Disc Preparation

The sterile discs were impregnated with solvent extracts of absorbent paper. It was most convenient to use Whatman No.1 filter paper for preparing the discs. Dried disc size was 6 mm diameter.

Tested Microorganisms

Antimicrobial activity of various solvents were investigated against gram positive bacteria *viz., Staphlococcus aureus, Bacillus cereus* and gram negative bacteria *viz., Serratia marcescens* and *Klebsiella pneumoniae*. Fungal pathogen *viz., Candida albicans* and *Aspergillus niger*. These human pathogens were purchased from Department of Microbiology, K.A.P Viswanatham medical college, Tiruchirappalli, Tamil Nadu

Procedure

Sterile liquid nutrient agar/potato dextrose agar medium (pH 7.4 ± 2) was transferred (10-15ml) into each sterile petriplates. These growth media an important role in the determination of the antimicrobial assay after solidification, 100µl of suspension containing 108 CFU/ml of each test bacteria/fungi were spread over nutrient agar/potato dextrose agar plates. The soaked discs paper placed on the inoculated agar. Negative controls were prepared in using the same solvents employed. Chloramphenicol (30mcg/disc)/Amphotericin (100mcg/disc) were used as positive reference control to conclude the sensitivity of the solvent extracts on each microorganism. The inoculated plates were incubated 37°C at 24hrs for bacteria culture/ 37°C at 48-72hrs for

fungal culture. Antimicrobial activity was assessed by measuring the diameter of the inhibition zones. Each assay was conducted in triplicate.

Disc diffusion method

In vitro antimicrobial activity of the leaf and stem extracts of *Andrographis echioides* were investigated by standard disc diffusion method against selected human pathogens. Among various solvent like ethanol, methanol, acetone, ethyl acetate, chloroform, petroleum ether and aqueous extracts were used for this experiment. In addition, the inhibition zones formed by standard antibiotic disc (Chloramphenicol 30 mcg/disc and amphotericin 100mcg/disc) and those filter paper discs injected with selected solvents (negative controls). The diameter of inhibition zones for each of the samples were paralleled with standard antibiotics. It was noted that the inhibition zones of the tested plant extracts to be either less than or greater than or equal to the inhibition zones of standard antibiotics.

Statistical analysis

Agar disc diffusion assay was achieved in replicates under strict aseptic conditions to ensure consistency of all conclusions. Data of all experiments were statistically analyzed and expressed as Mean ± Standard Deviation.

Result and Discussion

The results were observed on ethanol, methanol, acetone, ethyl acetate extracts exhibited highest degree of inhibition followed by chloroform, petroleum ether and aqueous solvent extracts. The chloroform extract was showed low degree of inhibition against all the tested pathogens. The antimicrobial activity found in the plant extracts have been attributed to some of the secondary metabolites (Giwa *et al*., 2010). In general, Gram-negative bacteria were more resistant to antibiotics than Gram-positive bacteria (Chowdhury *et al*., 2004). The resistance of Gram negative bacteria towards antibacterial substances is interrelated to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules. The membrane is also associated with the enzymes in the periplasmic space which are capable of breaking down the molecules introduced from outside (Shan *et al*., 2007). However, the Gram positive bacteria do not possess such outer membrane and cell wall structures (Kalamba and Kanicka, 2003). In this present investigation, all the extracts exhibited broad spectrum of activity expects petroleum ether and aqueous extract when the seven extracts were compared with each other that of standard antibiotic. The result shows in Ttable-1. The experiment made on ethanol leaf extract highest activity against *Staphylococcus aureus* (13.3±2.8), *Candida albicans* (12.3±1.3), *Klebsiella pneumoniea* (12.3±1.1), *Serratia marcescens* (11±1) and *Bacillus cereus* (10.3±0.5). The methanol leaf extracts also highest activity observed against *Staphylococcus aureus* (14±1.7), *Serratia marcescens* (13.6±1.1) *Candida albicans* (13.3±1.1) *Klebsiella pneumniea* (12.3±2.5) and *Bacillus cereus* (10.6±1.1). Acetone leaf extract pointed out highest activity against *Serratia marcescens*(12±1.7), *Bacillus cereus* (11.6±2.0) and *Candida albicans* (10.4±1.0) and the lowest activity against *Staphylococcus aureus* (7.6±0.5) and *Klebsiella pneumoniea* (5.6±1.7). The methanol and ethyl acetate extracts of *Andrographis lanata* showed the interesting antimicrobial activity (Chowdhury *et al.,* 2002). In this present study, the ethyl acetate leaf extract showed uppermost activity against all the tested pathogens such as *Klebsiella pneumoniea* (23±1.7), *Bacillus cereus* (20±2), *Staphylococcus aureus* (15.6±2.0), *Serratia marcescens* (13.3±2.8), and *Candida albicans* (12.5±1.1). The lowest zone of inhibition was observed against *Aspergillus niger* (7±0). The chloroform leaf extract showed minimal zone of inhibition against *Serratia marcescens* (8.3±1.1) and *Klebsiella pneumoniea* (8.3±1.7) and it has no activity observed against *Staphylococcus aureus, Bacillus cereus,* and *Candida albicans*. Whereas no antimicrobial activity on petroleum ether and aqueous extract against all the tested bacteria and fungi pathogens and it has minimal zone of inhibition was observed on aqueous leaf extract against *Staphylococcus aureus* (9±0.5). All the selected solvent extracts have no antimicrobial activity against *Aspergillus niger* but the ethyl acetate extract minimal zone of inhibition was observed against *Aspegillus niger*. Revathi *et al.,* (2012) conveyed that the antimicrobial activity of *Andrographis serpyllifolia* extract from root, stem and leaf. Comparable result was found from the antibacterial activities of *Tribulus terrestries* (Vishal Kumar Deshwal, 2012) and *Andrographis paniculata* (Vijyakumar Arul Dass and Kalaichelvan, 2012). In present study, the antimicrobial activity of *A. echioides* stem extract of ethanol showed maximum zone of inhibition was observed against *Candida albicans* (18±1.5),

Klebsiella pneumoniea (13.3±1.5), *Serratia marcescens* (13±1.7), *Bacillus cereus* (10.6±1.0) and *Staphylococcus aureus* (10.3±1.5). The methanol extract also showed highest activity observed against *Candida albicans* (15.3±1.4), *Klebsiella pneumoniea* (14.3±2.0), *Serratia marcescens* (11.6±1.5), *Staphylococcus aureus* (10.6±1.5) and *Bacillus cereus* (10.6±1.1). Then the antimicrobial activity of stem extract by acetone showed highest zone of inhibition noted against *Klebsiella pneumoniea* (13±1.0), *Bacillus cereus* (12.6±1.5) and *Serratia marcesens* (11.6±1.2) and their the minimal activity observed against *Candida albicans* (8.3±1.5) and *Staphylococcus aureus* (7.3±0.5).

In this present experiment, the ethyl acetate stem extract showed highest antimicrobial activity against all the tested human pathogenic bacteria and fungi such as *Bacillus cereus* (17.3±1.8), *Candida albicans* (16.3±1.8), *Serratia marcescens* (15.3±2.0), *Klebsiella pneumoniea* (14.6±1.5) and *Staphylococcus aureus* (12.6±1.5). And their lowest activity observed against *Aspergillus niger* (7±1.0). The result shows in (Table-2). The chloroform stem extract showed lowest zone of inhibition was observed against *Klebsiella pneumoniea* (9±1), *Serratia marcescens* (8±1) and *Staphylococcus aureus* (5±1.3). It has no activity observed on chloroform stem extract against *Bacillus cereus*, *Candida albicans* and *Aspergillus niger*. Whereas no activity on petroleum ether and aqueous extracts against all the tested pathogenic bacteria and fungi and it has lowest zone of inhibition observed on aqueous stem extract against *Staphylococcus aureus* (8.3±1.0). The antibacterial potential of another species of this family (Acanthaceae) has been informed (Abubacker and Vasantha, 2010; Sukesh *et al*., 2011). Alternative species of this genus, *Andrographis* like *Andrographis lineata, Andrographis echioides* and *Andrographis paniculata* were described as value antimicrobial medicines (Santhi *et al*., 2006; Abubacker and Vasantha, 2010; Vijayakumar Arul Doss and Kalaiselvan, 2012). In another investigation ethanol, chloroform, acetone and ethyl acetate extracts of *Solanum trilobatum* was found to be worthy antibacterial properties (Natarajan and Kamalanathan, 2012). Alternative species of this genus, *A. paniculata* has been vastly studied and recognized. Acetone, ethanol, methanol, aqueous and hexane extract of *A. paniculata* were highly active against selected bacterial species like *Staphylococcus aureus, Streptococcus pyogenes, Bacillus cereus, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis* (Vinothkumar *et al*., 2010).

Table 1: Antimicrobial screening of leaves extract *Andrographis echioides* (L) on human pathogens (Disc diffusion method)

Test bacteria	Inhibition zone diameter in mm (mean ± SD)														
	Ethanol		Methanol		Acetone		Ethyl acetate		Chloroform		Petroleum ether		Aqueous		Positive Control
	Experiment (30 µg/disc)	N	Experiment (30 µg/disc)	N	Experiment (30 µg/disc))	N	Experiment (30 µg/disc)	N	Experiment (30 µg/disc)	N	Experiment (30 µg/disc))	N	Experiment (30 µg/disc))	N	C * (100 mcg/disc)
Positive bacteria															
Staphylococcus aureus	13.3±2.8	-	14±1.7	-	7.6±0.5	-	15.6±2.0	-	-	-	-	-	9±0.5	-	26.6±0.5
Bacillus cereus	10.3±0.5	-	10.6±1.1	-	11.6±2.0	-	20±2	-	-	-	-	-	-	-	21±0
Negative bacteria															
Serratia marcescens	11±1.7	-	13.6±1.1	-	12±1.7	-	13.3±2.8	-	8.3±1.1	-	-	-	-	-	28±0
Klebsiella pneumonia	12.3±1.1	-	12.3±2.5	-	5.6±1.5	-	23±1.7	-	8.3±1.1	-	-	-	-	-	12.3±0.5
Fungi															
Candida albicans	12.3±1.3	-	13.3±1.1	-	10.4±1.0	-	12.5±1.1	-	-	-	-	-	-	-	12.6±1.5
Aspergillus niger	12.3±1.1	-	13±1.2	-	12.3±1.1	-	14.2±1.1	-	-	-	-	-	-	-	16.2±0.5

'–' represents as 'no inhibition'
N – Negative control (Respective solvent 30 µg/disc)
C* – Positive control (Amphotericin 100mcg/disc)*

Table 2: Antimicrobial screening of stem extract of *Andrographis echinoids* (L) on human pathogens (Disc diffusion method)

Inhibition zone diameter in mm (mean ± SD)															
Test bacteria	**Ethanol**		**Methanol**		**Acetone**		**Ethyl acetate**		**Chloroform**		**Petroleum ether**		**Aqueous**		**Positive Control**
	Experiment (30 µg/disc)	**N**	**Experiment (30 µg/disc)**	**N**	**Experiment (30 µg/disc))**	**N**	**Experiment (30 µg/disc)**	**N**	**Experiment (30 µg/disc)**	**N**	**Experiment (30 µg/disc))**	**N**	**Experiment (30 µg/disc))**	**N**	**C * (100 mcg/disc)**
Positive bacteria															
Staphylococcus aureus	10.3±1.5	-	10.6±1.5	-	7.3±0.5	-	12.6±1.5	-	5±1.3	-	-	-	8.3±1.0	-	26.6±0.5
Bacillus cereus	10.6±1.0	-	10.6±1.1	-	12.6±1.5	-	17.3±1.8	-	-	-	-	-	-	-	21±0
Negative bacteria															
Serratia marcescens	13±1.7	-	11.6±1.5	-	11.6±1.2	-	15.3±2.0	-	8±1	-	-	-	-	-	28±0
Klebsiella pneumonia	13.3±1.5	-	14.3±2.0	-	13±1.0	-	14.6±1.5	-	9±1	-	-	-	-	-	12.3±0.5
Fungi															
Candida albicans	18±1.5	-	15.3±1.4	-	8.3±1.5	-	16.3±1.8	-	-	-	-	-	-	-	12.6±1.5
Aspergillus niger	-	-	-	-	-	-	7±1.0	-	-	-	-	-	-	-	13±0.5

'–' represents as 'no inhibition'
N – Negative control (Respective solvent 30 µg/disc)
C* – Positive control (Amphotericin 100mcg/disc)*

Conclusions

This medicinal plant *Andrographis echioides* leaf and stem result showed potential antimicrobial activities against the tested human pathogenic bacteria and fungi. However, this medicinal plant species may be exposed to detailed phytochemical and pharmacological studies in order to find out new remedies against bacterial and fungal strains.

Reference

Abubacker, M.N., Vasantha, S. 2010. Antibacterial activity of ethanolic leaf extracts of *Andrographis paniculata* Nees (Acanthaceae) and its bioactive compound Andrographolide. *Drug Invention Today*, 2(10): 440-442.

Agnel Ruba, A., Mohan, V.R.., 2016. *In Vitro* Antioxidant Potential of Whole Plant of *Andrographis echioides* (L.) Nees (Acanthaceae). *International Journal of Pharmacy and Pharmaceutical Sciences*, 8(6); 2016.

Anonymous. *Andrographis echioides* (L.) Nees in Wall., Pl. Asiat. Rar. 3: 117. 1832; Hook. f., Fl. Brit. India 4: 505. 1884;

Chowdhury AA, Islam MS. 2004. Antibacterial activity of Trema orientalis. *Dhaka Univ J Pharam Sci*; 3(1-2):115-117.

Chowdhury D, Sayeed A, Islam A, Bhuiyan MSA and Khan GRMAM. 2002. Activity and cytotocicity of *Aerva lanata*. Fitoterapia; 73:92-94.

Cowan, M.M., 1999 Plant products as antimicrobial agents, Clinical Microbiolo. Review. 12(4): 564-582.

De-Yang Shen, Shin-Hun Juang, Ping-Chung Kuo, Guan-Jhong Huang, Yu-Yi Chan, Amooru G. DamuandTian-Shung Wu,. 2013. Chemical Constituents from Andrographis echioides and Their Anti-Inflammatory Activity. *Int. J. Mol. Sci*, 14; 496-514.

Farnsworth NR, Soejarto DD. 1991. Global importance of medicinal plants. In: Akerele O, Heywood V, Synge H, editors. The conservation of medicinal plants. Cambridge (UK): Cambridge University Press; p. 25–51.

Gambal JS.Flora of the presidency of Madras.Botanical survey of India. Calcutta: 1956; 1051.

Gijtenbeek, JMM, Vanden Bent MJ and Vecht CJ. Cyclosporin neurotoxicity. *Journal of neurotoxicity*, 1999; 246: 339-46

Johnson WC., William WO. 2002. Benefits morbidity and mortality associated with longterm administration of oral anticoagulant therapy to patients with peripheral arterial bypass procedures: A prospective randomized study. *Journal of vascular surg*., 35(3): 413- 421.

Giwa OE, Seyifunmi OE, Adewumi BL, Adebote VT, Aladejimokun AO. 2010. Screening of antimicrobial ethanolic extract of *Peristrophe bicalyculata*. Ethnobot Leaflets; 14: 766-773.

Kalamba D, Kanicka A. 2003. Antibacterial and antifungal properties of essential oils. *Curr Med Chem;* 10: 813-829.

Kumaraswamy M V, Kavitha H U, Satish S (2008). Antibacterial Evaluation and Phytochemical Analysis of *Betula utilis* D. Don against Some Human Pathogenic Bacteria. *Journal of Agricultural Sciences* 4 (5): 661-664

Micke O, Hubner J, Munstedt K. 2009. Ayurveda. Der Onkologe 15, 792-798.

Nadkarni AK, Nadkarni KM., *Indian MateriaMedica*, Vol. 1. Bombay: Popular Prakashan, 1976.

Natarajan, D., Kamalanathan, D. 2012. Screening of antibacterial potential of leaves and leaf derived callus extracts of *Solanum trilobatum* L. an important medicinal plant. Journal of Pharmacy Research, 5:825-827.

Pandi Kumar P, Ayyanar M, lgnacimuthu S. 2007. Medicinal plants used by Malasar tribes of Coimbatore district, Tamil Nadu. *Indian J Tradit Knowl*, 6, 579-82.

Patwardhan B, Warude D, Pushpangadan P. Bhatt N. 2005. Ayurveda and traditional Chinese medicine: A comparative overview. *Evidence-Based Complementary and Alternative Medicine* 2, 465- 473.
Qadrie ZL, Beena Jacob, Anandan R, Rajkapoor B, Rahamathulla M. Antibacterial activity of ethanol extracts of *Indoneesiella echioides* evaluated by the filter paper disc method. *Pak J Pharm Sci,* 2009; 22: 123-5.

Revathi, S.L., Sureshkumar, P., SudarshanaDeepa, V., Senthilkumar, J., Anitha Janet Roshini, Y. 2012. Antimicrobial activity of *Andrographis serpyllifolia* (Vahl) Wight.International *Journal of Pharmaceutical Sciences and Health Care*. 1(2):17-31.

Basu, S.K, Rupeshkumar .M, Kavitha .K, 2009. Studies Othe Ati-Iflammatory, Aalgesic Ad Atipyretic Properties *of Pavoia Zeylaica,* Pharmacologyonline 1; 1144-1153.

Santhi, R., Alagesaboopathi, C., Rajasekarapandian, M. 2006. Antibacterial activity of *Andrographis lineata* Nees and *Andrographis echioides* Nees of Shevaroy Hills of Salem district, Tamilnadu. *Advances in Plant Sciences*, 19: 371-375.

Shan B, Cai YZ, Brooks JD, Corke H. 2007. The *in vitro* antibacterial activity of dietary spice and medicinal herb extract. *Int J Food Microbiol*, 117: 112-119.

Shengji P. 2001. Ethnobotanical approaches of traditional medicine studies: some experiences from *Asia. Pharm Bot*.39:74–79

Sukesh, K., Shafi Thompson, T., Densingh, J. 2011. Phytochemical investigation and antibacterial activity of *Gymnema sylvestre* and *Andrographis paniculata* from Western Ghats. *International Journal of Phytomedicine*., 3: 254 -260.

Vijayakumar Arul Doss, Kalaichelvan, P.T. 2012. *In vitro* Antimicrobial and antioxidant activity screening of *Andrographis paniculata* leaf ethanol extract in Tamilnadu. *Int J Pharm Pharm Sci*., 4 (1): 227-229.

Vinothkumar, P., Sivaraj, A., Syed Zameer Ahmed, K., Sivamani, P., Devi, K., Senthilkumar, B. 2010. Evaluation of antibacterial activities of *Andrographis paniculata* leaf extract against selective gram positive and gram negative species by *in vitro* methods. *Journal of Pharmacy Research*., 3(7):1513-1515.

Vishal Kumar Deshwal. 2012. *In Vitro* Antibacterial Acti/Vity of Water And Ethanol Extract Of *Tribulus Terrestris* on The Growth of Pseudomonas Aeruginosa By Disc Diffusion Test. International Journal of Plant, Animal and Environmental Sciences, 2(2); 235-238.

Wasshausen, D.C. and Wood, J.R.I. (2004) Acanthaceae of Bolivia. Contributions from the *United States National Herbarium* 49: 1–152.