Medwell Online
Research Journal of Biological Sciences 2 (4): 419-423, 2007
© Medwell Journals, 2007

Evaluation of *Lawsonia inermis* Linn. (Sudanese Henna) Leaf Extracts as an Antimicrobial Agent

M.A. Abdulmoneim Saadabi
School of Life Sciences, Faculty of Science and Technology, Al-Neelain University, Khartoum, Sudan

Abstract: Leaf samples of *Lawsonia inermis* were collected from Dammar reigon, north of Sudan to examine their antimicrobial potential. Water, methanol and chloroform crude extracts in different concentrations were obtained and bioassayed *in vitro* for its bioactivity to inhibit the growth of 6 human pathogenic fungi and 4 types of bacteria. The differences in bioactivity of the 3 types of extracts were analyzed. Despite extreme fluctuations in activity, the extract of water was clearly superior. Then followed by methanol, while chloroform extract showed the least. The growth of all pathogens was inhibited to varying degrees by increasing the concentration of the extract. Phytochemical analyses showed the presence of anthraquinones as major constituents of the plant leaves and are commonly known to posses antimicrobial activity. These results confirm the antibacterial and antifungal activity of henna leaves and support the traditional use of the plant in therapy of bacterial infections. The possibility of therapeutic use of Sudanese henna as antimicrobial agents is worthy of note.

Key words: Henna, *Lawsonia inermis*, medicinal plants, antimicrobial activity, antifungal activity, Sudan

INTRODUCTION

Some bacteria and fungi are extremely pathogenic causing serious human infections. The discovery of antibiotics to combat these pathogens marked a resolution in the 20th century (Evan, 1992). Unfortunately, because of the inappropriate use of antibiotics in human and veterinary medicine, certain strains of bacteria and fungi developed the ability to produce substances which block the action of antibiotics or change their target or ability to penetrate cells (Ali *et al*., 1995). Therefore, disease-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. Tuberculosis, gonorrhea, malaria and childhood ear infections are just a few of the diseases that have become hard to treat with antibiotics. However, a large part of the problem is due to our increasing use and misuse of existing antibiotics in human and veterinary medicine and in agriculture (Muhammad and Muhammad, 2005). To substitute synthetic antibiotics, many of today modern and effective drugs have their origin in traditional folk medicine (Natarajan *et al*., 2003). Plants have been used to treat human, animals and plant diseases from time immemorial. Also herbal medicines have been known to man for centuries (Goun *et al*., 2003; Misra and Sahu, 1977). Therapeutic efficacy of many indigenous plants for many disorders has been described by practitioners of traditional medicine (Al Maqbool *et al*., 1985, 1988; Iqbal *et al*., 2002; Khattak *et al*., 1985). The henna plant *Lawsonia inermis* Linn. is one such plant known since with healing attributes and is now the subject of intense scientific study (Singh and Singh, 2001; Azaizeh *et al*., 2003; Mitscher *et al*., 1972; Hanke and Talaat, 1961; Cajkovac *et al*., 1996; Malekzadeh and Shabestari, 1989). The plant belongs to the family Lythraceae and in Sudan is traditionally used to develop a red or black coloring to hands, feet and hair in some occasions such as weddings and religious festivals. The plant is planted in home gardens as hedges and as ornamental. It is a perennial shrub branching profusely and reaching a height of up to six meters. The leaves of the plant are small, lanceolate, dark-green and glabrous, opposite, with very short petioles. Flowers are small, white or pale pink colored and fragrant. The plant leaf contains a red orange color component, lawsone (2-hydroxy-1, 4-Napthoquinone). The capsulated fruits are borne in clusters, green in color. Henna is believed to have a cooling effect on the body and the paste is used to bring down fever. As a medicinal plant, because of its attributed antibacterial, antifungal, antiamoebiasis, astringent, antihemorrhagic, hypotensive and sedative effects (Khattak *et al*., 1985) it has also been used as a folk remedy against headache, jaundice and leprosy (Information by folk medicine herbalist from Khartoum). According to phytochemical analysis of henna, powdered leaves contain about 0.5-1.5% lawsone, the chief constituent responsible for the dyeing properties of the plant. Henna also contains mannite, tannic acid, mucilage, gallic acid and napthoquinone

(Ahmed *et al*., 2000; Rosenberg, 1999; Vardamides *et al*., 2001; Khan *et al*., 1991). Antimicrobial properties of *Lawsonia inermis* were investigated by several workers from a round the world (Dixit *et al*., 1980; Habbal Omer *et al*., 2005; Bhomick and Chooudhay, 1982; Natarajan and Lalithakumar, 1987).

The paucity of pharmacological and chemical data of Sudanese henna plant prompted an investigation into its antimicrobial activity. Therefore, the present study was planned to find out the antimicrobial activities of henna leaves and their efficacy against different fungal and bacterial strains.

MATERIALS AND METHODS

Plant material: The study was carried out at the Department of Biology at Abha, during January to December 2005. Henna plant *Lawsonia inermis* Linn. leaves samples used in this study were collected from Dammar city, North of Sudan. It was identified and authenticated by the Department of Botany, University of Khartoum, Sudan. A voucher specimens were deposited at Biology Departmental Herbarium. Fresh leaves were dried in shade, then were ground to powder.

Preparation of extracts: Ten gram of the coarsely powdered plant material were successively Soxhlet extracted with $CHCL_3$ and MeOH for 24 h. The extracts were evaporated under vacuum and the residues were separately dissolved or suspended in the same extracting solvent (10 mL) and kept in refrigerator till use. In addition, water extracts were prepared by adding distilled water to 10 g of coarsely powdered plant material in a conical flask and left to soak overnight. The residue was then filtered and the final volume was adjusted to 10 mL with distilled water and the solution used immediately.

Fungal strains: Six fungal species were obtained from Abha General Hospital, Asir, KSA. The fungi were isolated from clinical cases. These fungi were *Epidermophyton floccosum, Microsporum audouinii, Trichophyton rubrum, Trichophyton concentricum, Trichophyton tonsurans* and *Candida albicans* (Table 1). Each organism was cultured on Sabouraud, s dextrose agar medium (Oxoid) incubated at 25°C for 7 days, to obtain inoculums for testing.

Bacterial strains: Four types of bacteria namely *Staphyllococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* were used. The bacteria was cultured on nutrient broth (Oxoid) at 37°C for 24 h.

Determination of antifungal bioassay: Sterile, filter paper discs of 6 mm diameter were impregnated with about 0.1 mL $disc^{-1}$ of extract which have been dissolved

Table 1: Comparative bioactivity of *Lawsonia inermis* Linn. leaf extracts against different fungal isolates*

Extract type	Concentration ($mg\ mL^{-1}$)	Fungal isolates *Ef*	*Ma*	*Tr*	*Tc*	*Tt*	*Ca*
Water	25	14.25±0.12	13.21±0.55	13.20±0.05	14.00±0.22	14.44±0.52	14.38±0.78
	50	15.32±1.03	14.12±1.13	15.23±1.15	15.18±1.25	15.00±1.05	14.95±0.28
	75	15.94±0.42	15.82±0.03	16.18±1.65	15.95±0.35	15.75±0.85	15.11±0.48
Methanol	25	13.25±1.05	11.41±0.65	10.51±0.45	10.95±1.05	12.65±1.00	14.00±0.11
	50	14.28±0.75	12.37±0.15	11.00±1.98	11.33±1.08	12.73±0.08	14.89±0.77
	75	14.95±0.55	13.67±1.16	12.27±1.16	12.00±1.99	12.95±0.99	15.00±0.44
Chloroform	25	12.77±0.65	11.22±0.35	11.11±0.75	11.00±0.25	13.00±0.65	13.11±0.55
	50	14.20±1.15	12.17±1.22	11.95±0.98	11.45±0.98	13.15±0.27	13.68±0.88
	75	14.95±0.55	14.20±1.00	12.37±1.44	12.50±1.04	13.51±0.14	13.98±0.66
Nystatin (reference drug)	25 $ug\ mL^{-1}$	16.23±0.85	16.75±0.41	16.95±0.76	17.75±0.61	16.22±0.41	16.00±0.01

*Data are presented as mean±SD of zone of inhibition (mm); inhibition zones are the mean of three replicates; *Ef =Epidermophyton floccosum, Ma =Microsporum audouinii , Tr= Trichophyton rubrum , Tc= Trichophyton concentricum, T t= Trichophyton tonsurans, Ca= Candida albicans*

Table 2: Influence of Henna (*Lawsonia inermis* Linn.) leaf water extracts on the growth of different fungal dermatophytes*

Clinical fungal isolates	Mycelial fresh weight (g) grown on Czapek-Dox medium 0 (control)	25 $mg\ mL^{-1}$	50 $mg\ mL^{-1}$	75 $mg\ mL^{-1}$	D.S.D. (at 1%)	L.S.D. (at 5 %)
Epidermophyton floccosum	12.00	6.95	5.16	4.50	1.333	0.652
Microsporum audouinii	14.25	5.22	4.75	3.00	1.100	1.010
Trichophyton rubrum	13.00	7.00	5.98	5.00	1.999	1.300
Trichophyton concentricum	11.00	6.50	6.00	6.00	1.033	1.011
Trichophyton tonsurans	10.75	7.00	5.50	3.75	2.001	1.751
Candida albicans	9.35	4.75	4.00	3.00	1.231	0.541
C.D.at 1%	1.909	1.810	1.980	2.000		
C.D.at 5 %	1.460	1.125	1.333	1.400		

*Values are means of 5 replicates

Table 3: *In vitro* antibacterial activity of *Lawsonia inermis* Linn. leaf extracts *

Extract type	Zone of inhibition (mm) B.s	S.a	E.c	P.a
Water	16b	19a	16a	18a
Methanol	14a	16b	17b	16b
Chloroform	14a	13c	14c	15b
Ampicillin 40 ug mL^{-1}	20	22	24	16

*B.s=*Bacillus subtilis*; S.a=*Staphyllococcus aureus*; E.c = *Escherichia coli*; P.a = *Pseudomonas aeruginosa*; concentration of extracts 0.1 mL cup^{-1} (100 mg mL^{-1}); inhibition zones are the mean of three replicates. Means followed by the same letter in columns are not significantly different ($p<0.05$)

Table 4: Phytochemical analysis of *Lawsonia inermis* Linn. leaf samples

Constituent	Level*
Mannite	++
Tannic acid	+++
Gallic acid	++
Naphtaquinone (hennotannic acid)	+++
Crysophanic acid	+++
Anthraquinones	+++
Mucilage	+++
Sterols and/or triterpenes	±
Cyanogenic glycosides	+
Flavonoids	-
Tannins	-
Saponins	-

*:+ = Low concentration ++ = Medium concentration, +++ = High concentration, ± = traces, - = Not detectable

in Dimethyl Sulphoxide (DMS) and placed in duplicates onto the Sabouraud' s dextrose agar plates, seeded with 0.2 mL of fungal suspension. The plates were then incubated at 25°C for 10-14 days (Ugarte *et al*., 1987; Iqbal *et al*., 2002). The zone of inhibition around each disc was measured in mm. The results are presented as mean±SD of zone of inhibition. Nystatin (25 μg $disc^{-1}$) was used as a reference standard drug for comparison (Table 1). To determine the influence of Henna leaf water extract on the growth of six different fungal dermatophytes, aliquots of different dilutions of the extracts (25, 50 and 75 mg mL^{-1}) were separately added in 175 mL Czapek-Dox liquid medium in conical flask. Flasks containing Czapek-Dox medium alone (200 mL) served as control. Flasks were then inoculated with *Epidermophyton floccosum*, *Microsporum audouinii*, *Trichophyton rubrum*, *Trichophyton concentricum*, *Trichophyton tonsurans* and *Candida albicans* (Table 2) then incubated at 25°C. Each set of treatments was replicated 5 times. After 15 days the fungal mats were harvested through Whatman filter paper No. 2 (which removes mycelium), then gently pressed between the folds of blotting paper to remove the excess amount of water and weighed to determine the mycelial fresh weight (Table 2).

Antibacterial bioassay: Each leaf extract (water, methanol and chloroform) was tested against the four types of bacteria using the cup-plate agar diffusion method(Groove and Randall, 1955) and the inhibition zones were measured. The means of the diameters of the inhibition zones are reported in Table 3.

Phytochemical screening: Phytochemical screening was carried out for henna leaf samples using the method adopted by Crombie *et al.* (1990) and phytochemical analysis results of *Lawsonia inermis* leaves were reported in Table 4.

RESULTS AND DISCUSSION

As a general rule, plant is considered active against both fungi and bacteria when the zone of inhibition is greater than 6 mm (Muhammad and Muhammad, 2005). Results presented in Table 1 and 3 indicated that all of the 3 tested leaf extracts of henna plant *(Lawsonia inermis)* at different concentrations significantly ($p = 0.05$) suppressed the growth of the tested pathogenic fungi and bacteria at varying degrees. Extracts of water was clearly superior in bioactivity as compared to that of methanol and chloroform. The maximum inhibition zone was found in 75 mg mL^{-1} water extract concentration and it was 16.18±1.65 mm in the fungal isolate of the pathogen *Trichophyton rubrum*, while the least inhibition zone in the same concentration was 15.11±0.48 mm for the pathogen *Candida albicans*. The activity increased with increasing the extract concentration. Even in the lowest concentration of water extract i.e., 25 mg mL^{-1}, all of the fungal isolates showed substantial inhibition in their respective growth. The minimum inhibition zone in 25 mg mL^{-1} concentration was observed in *Trichophyton rubrum* with 13.20±0.05 mm inhibition zone (Table 1) as compared to 16.00±0.01 mm in *Candida albicans* at the same concentration for the reference nystatin antibiotic (Table 1). The maximum inhibitory effect for methanol extract at 25 mg mL^{-1} was observed also against *Candida albicans* where the zone of inhibition was 14.00±0.11 mm against standard antibiotic (16.00±0.01 mm) and a minimum activity was observed against *Trichophyton rubrum* (10.51±0.45 mm). The chloroform extract of the plant showed maximum activity against the *Candida albicans* where the zone of inhibition was 13.11±0.55 mm compared to that of the reference drug (16.00±0.01 mm) and the weakest activity was observed against *Trichophyton concentricum* (11.00±0.25 mm) in the same concentration (25 mg mL^{-1}). Results also showed that the growth in culture media of the different clinical fungal isolates was suppressed when the water leaf extract of henna was used in different concentrations (Table 2). Percent reduction in mycelial weight of the different tested fungal species was directly correlated to the concentration of the leaf extract

(Table 2). The maximum reduction in mycelial weight (3.00 g) was observed in 75 mg mL^{-1} water extract concentration in *Microsporum audouinii* and *Candida albicans* followed by *Trichophyton tonsurans* (3.75 g) and *Epidermophyton floccosum* (4.50 g). The minimum reduction, on the other hand, was brought about by the water extract concentration 75 mg mL^{-1} for the pathogen *Trichophyton concentricum* (6.00 g). However, the reduction in mycelial weight is decreased when the concentration of the extract was increased with no significant differences between the three used water extracts concentrations of each treatment. Even in 25 mg mL^{-1} as lowest concentration the reduction in mycelial weight of the different tested fungi was significant over controls (Table 2).

Results obtained from *in vitro* antibacterial activity showed that the three types of henna extracts has a substantial inhibitory effects against the four tested bacterial strains (Table 3). Still the water extract was superior in suppressing the bacterial growth, followed by methanol and chloroform extracts. The maximum inhibition zone was observed against *Staphyllococcus aureus* (19 mm) followed by *Pseudomonas aeruginosa* (18mm) and 16mm for both *Bacillus subtilis* and *E. coli*. The weakest activity was observed in chloroform extract with a maximum zone of inhibition 15 mm observed against *Pseudomonas aeruginosa* and a minimum zone of inhibition 13 mm in *Staphyllococcus aureus* (Table 3). This data is in close agreement with previous reports elsewhere using the same plant (Bonjar, 2004; Muhammad and Muhammad, 2005).

Furthermore, the leaves of the plant were phytochemically screened and the results are shown in Table 4 and Fig. 1 and 2. The leaves of the plant showed the presence of tannic acid, naphtaquinone (hennotannic acid), crysophanic acid, anthraquinones and mucilage in high levels concentration. Other constituents such as mannite, gallic acid were found in a moderate concentration while cyanogenic glycosides were found in low concentration. Sterols and/or triterpenes were found in low detectable traces. Tannins and saponins were isolated from a few leaf samples of the plant in a medium concentration, hence the significance of this finding remains the area of further investigations as far as the chemical constituents of this plant is concerned. Also, It is not possible to make a direct correlation between the observed activity of the plant extracts *in vitro* and the actual effects when used *in vivo* for the diseases observed by the indigenous people and traditional healers. Therefore, it is important that the plant should also be further investigated to evaluate the significance

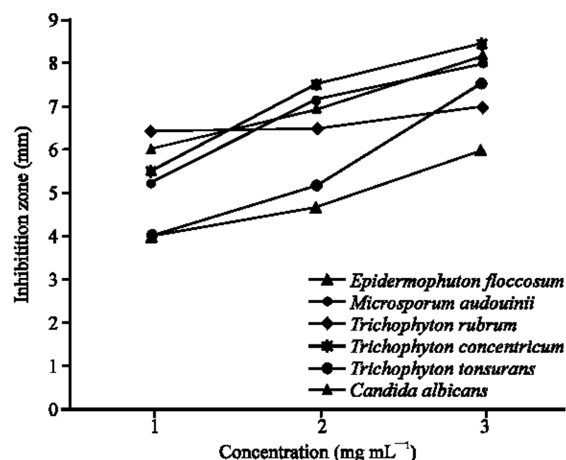

Fig. 1: Antifungal activity of anthraquinones derivatives from henna (*Lawsonia inermis*)

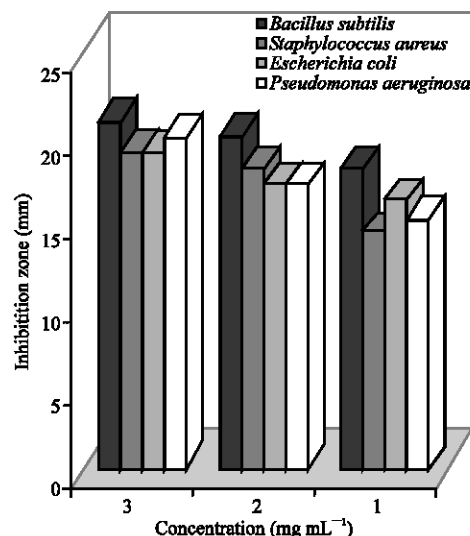

Fig. 2: Antibacterial activity of anthraquinones derivatives from henna (*Lawsonia inermis*)

of these extracts, clinical role and the medical system of indigenous people. Additional deep research is necessary to isolate and characterize their active compounds for pharmacological testing. The present study identifies Sudanese henna (*Lawsonia inermis L.*) as potential source of biological antimicrobial, since it showed a high activity against wide spectrum of bacteria and fungi which enables only human pathogenic fungi and bacteria to be killed without any side effects and/or bacterial resistance as current synthetic antibiotics are doing and this specificity appears as additional point in the natural antibiotics research.

REFERENCES

Ahmed S., A. Rahman, A. Alam, M. Saleem, M. Athar and S. Sultana, 2000. Evaluation of the efficacy of *Lawsonia alba* in the alleviation of carbon tetrachloride-induced oxidative stress. J. Ethnopharm., 69: 157-164.

Ali, B.H., A.K. Bashir and M.O.M. Tanira, 1995. Anti-inflammatory, antipyretic and analgesic effects of *Lawsonia inermis* L. (henna) in rats. Pharmacology, 51: 356-363.

Almaqbool, A.Z., A.K. Bashir, A. Farouk and A.K.M. Salih, 1985. Antimicrobial Activity of certain Sudanese plants used in Folkloric Medicine; Screening for Antimicrobial Activity (IV). Fitoterapia, 56: 331.

Almaqbool, A.Z., A.K Bashir and A.K.M Salih, 1988. Antimicrobial Activity of certain Sudanese plants used in Folkloric Medicine; Screening for Antimicrobial Activity (V). Fitoterapia, LIX:59-62.

Anand, K.K., B. Singh, D. Chand and B.K. Chandan, 1992. An evaluation of *Lawsonia alba* extract as hepatoprotective agent. Planta Medica, 58: 22-25.

Azaizeh, H., S. Fulder, K. Said and O. Khalil, 2003. Ethno-Botanical knowledge of local Arab practitioners in the Meddle Eastern region. Fitoterapia, 74: 98-108.

Bhomick, B.N. and B.K. Chooudhary, 1982. Antifungal activity of leaf extracts of medicinal plants on ***Alternaria alternata.*** Indian Botanical Reporter, 1: 164-165.

Bonjar, S.G.H., 2004. Screening for Antibacterial properties of some Iranian plants against two strains of ***Eschericia coli.*** Asian J. Plant Sci., 3: 310-314.

Cajkovac, M., L. Oremovic and V. Cajkovac, 1996. The effect of hair colors and perm products on the state of hair. Acta Pharm., 46: 39-49.

Crombie, L., W.M.L. Crombie and D.A. Whiting, 1990. Alkaloids of Khat (*Catha edulis*). Alkaloids, 39:139-164.

Dixit, S.N., H.S. Srivastava and R.D. Tripathi, 1980. Lawsone, the antifungal antibiotic from the leaves of *Lawsonia inermis* and some aspects of its mode of action. Indian Phytopathol., 31:131-133.

Evan, C.W., 1992. Trease and Evans Pharmacognosy, (13th Edn.), Bailliere Tindall, London, pp: 758-762.

Goun, E., G. Cunningham, D. Chu, C. Nguyen and D. Miles, 2003.Antibacterial and antifungal activity of Indonesian ethnomedical plants. Fitoterapia, 74: 592-596.

Groove, D.C. and W.A. Randall, 1955.Assay methods of Antibiotics. Medical Encyclopoedia, New York, pp: 24-55.

Habbal, O.A., A.A. AI-Jabri, A.H. El-Hag, Z.H. AI-Mahrooqi and N.A. Al-Hashmi, 2005. *In vitro* antimicrobial activity of *Lawsonia inermis* Linn. (henna). A pilot study on the Omani henna. Saudi Med. J., 26: 69-72.

Hanke, M. and S.M. Talaat, 1961. The biochemistry and physiology of henna (*Lawsonia alba*): Its use as a remedy for intestinal amoebiasis. Trans. Roy. Soc. Trop. Med. Hyg., 55: 56-62.

Iqbal Zafar, Shaheen Mussarat, H. Farrakh, B. Sheraz, I. Mohammad, Z. Shahida and A. Bashir, 2002. Antifungal properties of some indigenous plants from Peshawar Valley. Asian J. Plant Sci., 1: 708-709.

Khan, M.M., A. Ali, D.C. Jain, R.S. Bhakuni, M. Zaim and R.S. Thakur, 1991.Occurrence of some antiviral sterols in *Artemisia annua*. Plant Sci., 75: 161-165.

Khattak, S.G., S.N. Gilani and M. Ikram, 1985. Antipyretic Studies on some indigenous Pakistani Medicinal Plants. J. Ethno. Pharmacol., 14: 45-51.

Malekzadeh, F. and P.P. Shabestari, 1989. Therapeutic effects and *in vitro* Activity of an extract from *Lawsonia inermis*. J. Sci. Islamic Repub., 1: 7-12.

Misra, S.K. and K.C. Sahu, 1977.Screening of some indigenous plants for antifungal activity against dermatophytes. Indian J. Pharmacol., 9: 269-272.

Mitscher, L.A., R.P. Leu, M.S. Bathala, W.N. Wu and J.L. Beal, 1972. Antimicrabial agents from higher plants.1.Introduction, rationale and methodology. Lloydia, 35:157-166.

Muhammad, H.S. and S. Muhammad, 2005. The use of *Lawsonia inermis* Linn. (henna) in the management of burn wound infections. Afr. J. Biotech., 4: 934-937.

Natarajan, V., P.V. Venugopal and T. Menon, 2003. Effect of *Azadarichta indica* (neem) on the growth pattern of dermatophtyes. Indian J. Med. Microbiol., 21: 98-101.

Natarajan, M.R. and D. Lalithakumar, 1987. Leaf extracts of *Lawsonia inermis* as antifungal agent. Curr. Sci., 56:1021-1022.

Rosenberg, N.M., 1999. Antibacterial deodorizing compositions containing extracts of *Lawsonia inermis*. PCT. Int. Applied, pp: 37.

Singh, A. and D.K. Singh, 2001. Molluscicidal activity of *Lawsonia inermis* and its binary and tertiary combinations with other plant derived molluscicides. Indian J. Exp. Biol., 39: 263-268.

Ugarte, R., W. Quilhor, B. Diaz, A. Vera and D. Fieder, 1987. Atlvdade antimicrobiana *in vitro* del acido epiforelico L. Acta Farm. Bonaerense, 6: 65-69.

Vardamides, J.C., E. Dongo, A.E. Nkengfack, Z.T. Fomum, T.M. Ngando, B. Vogler and W. Kraus, 2001. Diterpenoids and Limonoids from the stem of *Pterorhachis zenkeri*. Fitoterapia, 72: 386-393.