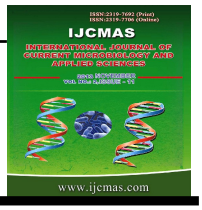
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 11 (2013) pp. 186-193**
http://www.ijcmas.com



Original Research Article

Antimicrobial activity of *Caesalpinia melanadenia* (Rose) Standl (Fabaceae)

Adriana Pichardo[1], Ximena Méndez[1], Griselda Alvarado[1], Pilar Ramirez[1], RocioSerrano[1], Marisol Avila[1], Samuel Meraz[1], Julieta Orozco[1], Ana García-Bores[2], J. Guillermo Avila[2] and Tzasna Hernández[1]*

[1]Laboratorio de Farmacognosia, UBIPRO, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla 54090, Edo. de México, México
[2]Laboratorio de Fitoquímica, UBIPRO, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla 54090, Edo.de México, México
****Corresponding author***

A B S T R A C T

Infusions of the aerial part of *Caesalpinia melanadenia* (Fabaceae) are used by the inhabitants of San Rafael Coxcatlán, Puebla (México) for the treatment of gastrointestinal, respiratory and skin diseases. The aim of this work was to investigate the antimicrobial activity of the aerial parts of *Caesalpinia melanadenia*, validate its use and contribute to the knowledge of medicinal flora from San Rafael municipality. Hexane and methanol partitions were used for antimicrobial test. Eight Gram positive, nine Gram negative bacteria and nine fungal strains were used in the antimicrobial assay. The methanol partition does not show any activity while hexane partition showed antibacterial activity against all the bacterial and fungal strains. The most sensitive strains were *E. feacalis* (MIC= 60 µg/mL), *S. pneumoniae* (MIC= 60 µg/mL), *S. epidermidis* (MIC= 250 µg/mL), *E. aerogenes* (MIC= 250 µg/mL) and *C. neoformans* (MIC = 125 µg/mL). The present study tends to confirm the use in folk medicine of *Caesalpinia melanadenia* against infectious diseases.

K e y w o r d s

Caesalpinia melanadenia; Antibacterial; Antifungal; Infectious diseases; Infusion.

Introduction

Caesalpinia melanadenia (Rose) Standl (Fabaceae) is commonly known as "Ixcanelillo". In México infusions of the aerial part are used in Mexican traditional medicine by the inhabitants of the village of San Rafael Coxcatlán, Puebla, for the treatment of gastrointestinal, respiratory and skin diseases. *C. melanadenia* is a shrub, endemic to Tehuacán-Cuicatán Valley, México (Argueta and Cano, 1994; Davila *et al*., 2002).

Some species of the *Caesalpinia* genus have been chemically examined and yielded diterpenes, triterpenes, flavanoids, quinones, and alkaloids; and also biological activities has been evaluated (antibacterial, antifungal, analgesic, anti-

inflammatory, etc.), including *C. ferrea*, (Carvalho *et al*., 1996), *C.pulcherrima* (Srinivas *et al*., 2003; Chakraborthy *et al*., 2009; Das *et al*., 2010), *C. sappan* (Pawar *et al*., 2008; Gan *et al*., 2010), *C. mimosoides* (Chanwitheesuk *et al*., 2005; Yodsaoue *et al*., 2011), *C. boduc* (Ata *et al*., 2009), *C. crista* (Das *et al*., 2010; Santnami and Yadava, 2011), *C. sappan* (Gan *et al*., 2010), and *C. digyna* (Srinivasan *et al*., 2010).

The genus *Caesalpinia* (Caesalpiniaceae) has more than 500 species, many of which have not yet been investigated for potential pharmacological activity (Zanin *et al*., 2012). *C. melanadenia* has no chemicals or biological studies. The aim of the study was to investigate the antimicrobial activity of the aerial part *C. melanadenia* (Fabaceae), validate its use and contribute to the knowledge of medicinal flora from San Rafael Coxcatlán, municipality.

Materials and Methods

Plant Material

Aerial parts of *C. melanadenia* were obtained in November 2011 from San Rafael Coxcatlán municipality. Dr. Edith López Villafranco of the IZTA Herbarium at the Factultad de Estudios Superiores Iztacala authenticated it. A voucher specimen was deposited in the IZTA herbarium (Voucher no. HCM15/2011).

Extract preparation and partitions

Aerial parts of *C. melanadenia* (182 g) were shade-dried at room temperature, ground into powder and sequentially extracted with methanol. The extract was filtered and successively concentrated. The methanol extract was redissolved in methanol and hexane was added to it in a separating funnel. After solvent-solvent extraction, the methanol phase was removed from the hexane phase. Both partitions, methanolic and hexanic were concentrated under low pressure (12.0 g and 3.5 g respectively) and kept in the dark at 4 °C until tested.

Microbial Strains

The following strains of bacteria were used: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 12398, *S. aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhy* ATCC 19430. *Bacillus subtillis, Staphylococcus epidermidis*, *Enterobacter aerogenes* were donated by FES-Cuautitlán. *B. subtilis, S. epidermidis, E.aerogenes* donated by the Clinical Analysis Laboratory of FES-Iztacala. *Streptococcus pneumoniae*, *Yersinia enterocolitica* were isolated from a clinical case and donated by Hospital Angeles (Metropolitano). *Vibrio cholera* isolated from a clinical case, *Vibrio cholerae* INDRE 206 (isolated from polluted water), *Vibrio cholerae* (clinical strain pertaining to 01 group, Inaba serotype, "El Tor" biotype, and enterotoxin producer). These strains were maintained at 4 °C in Mueller Hinton agar (Bioxon), submitted to sensitivity tests (multidiscs Bigaux) and were subcultured every month.

The yeasts tested were *Candida albicans* ATCC 10231, *C. albicans* ATCC 14065,*C. albicans* isolated from a clinical case donated by the Clinical Analysis Laboratory of FES-Iztacala, *C. albicans*, *C. glabrata*, *C. tropicalis* isolated from a clinical case and donated by Hospital Angeles (Metropolitano), *C. albicans* and *Criptococcus neoformans* donated by FES-Cuautitlán. The stock culture was maintained on Czapek Dox agar (Sigma).

Antibacterial Activity

The antibacterial activity was measured by the disk-diffusion method (Vanden Berghe and Vlietinck, 1991). The microorganisms were grown overnight at 37 °C in 10 mL of Mueller Hinton Broth (Bioxon). The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of McFarland no. 0.5 standard (1.0 x 10^8 CFU/mL) (Lennette *et al*., 1987). Petri dishes containing Mueller Hinton agar (Bioxon) were inoculated with these microbial suspensions.

Solutions of 200 mg/mL of each extract were prepared, disks of filter paper (Whatman no. 5) of 5 mm diameter were impregnated with 10 μL of each one (final doses per disk: 2000 μg of hexanic and methanolic partitions) and placed on the agar surface. Disks impregnated with hexane and methanol were used as negative controls. Disks with chloramphenicol (25 μg) were used as positive controls. The plates were incubated overnight at 37 °C and the diameter of any resulting zones of inhibition (mm) of growth was measured. Each experiment was performed in triplicates.

The estimation of the Minimal Inhibitory Concentration (MIC) was carried out by the broth dilution method (Vanden Berghe and Vlietinck, 1991). Dilutions of partitions from 2000 to 60 μg/mL were used. Test bacteria culture was used at the concentration of 10^5 CFU/mL. MIC values were taken as the lowest extract concentration that prevents visible bacterial growth after 24 h of incubation at 37 °C. Chloramphenicol was used as reference and appropriate controls with no extract were used. Each experiment was made three times.

Antifungal Activity

Yeast was assayed by the method described for bacteria, using Petri dishes containing CzapekDox Agar (20 mL), Nystatin (30 μg/disc) was used as reference and appropriate controls with no partitions were used. Each experiment was repeated three times. The estimation of the Minimal Inhibitory Concentration (MIC) and Minimal Fungicide Concentration (MFC) were carried out by the broth dilution method (VandenBerghe and Vlietinck, 1991). Dilutions of partitions from 2000 to 60 μg/mL were used. Test yeast culture was used at the concentration of 10^5 CFU/mL. MIC values were taken as the lowest partition concentration that prevents visible yeast growth after 24 h of incubation at 37 °C. Nystatin was used as reference and appropriate controls with no extract were used. Each experiment was made three times.

The inactivation broth death kinetic method was performed using appropriate concentrations of hexanic partition (corresponding to ½ MIC, MIC and MBC). Death kinetics expressed in $\log_{10}$ reduction time kills plots (Christoph *et al*., 2000).

Phytochemical screening

Preliminary phytochemical analysis was carried out using thin layer chromatography on silica gel plates developed with a mixture of toluene-ethyl acetate (93:7). Spots were revealed by the following spray-reagents: Dragendorff and Mayer reagent for alkaloids, vainillin-sulphuric acid for terpenes and flavonoids, and 2% methanol solution of α-naphtol with concentrated sulphuric acid for glicosides. The plates were dried, the presence of triterpenoids suggested by

violet spots and flavonoid by yellow or orange spots, mono and sesquiterpenes by blue-violet, red-violet, grey-blue or blue spots (Wagner *et al*., 2001; Sampietro *et al*., 2009).

Statistical analysis

All experiments were performed in triplicate. The mean and standard deviation of three experiments were determined. Statistical analysis of the differences between mean values obtained for experimental groups was done by analysis of variance (ANOVA multifactorial model), *p*-values of 0.001 or less were considered statistically significant.

Result and Discussion

The partitions yields were: hexanic 1.9 % w/w, and for methanolic 6.6 % w/w. The results obtained in the evaluation of the antimicrobial activity of the partitions of *C. melanadenia* are shown in Table 1. Only the hexanic partition showed antibacterial activity in eight Gram positive and nine Gram negative bacteria strains. The hexanic partition exhibited dose-dependent actions in all bacterial strains, which were statistically significant ($p < 0.05$).*E. feacalis* (MIC= 60 µg/mL), *S. pneumonia* (MIC= 60 µg/mL), *S. epidermidis* (MIC= 250 µg/mL) and *E. aerogenes* (MIC= 250 µg/mL) were the strains more sensitive to the hexanic partition effect. In general, Gram positive bacteria (MIC=60 - 750µg/mL) were more sensitive than the Gram negative ones (MIC=250 - 750 µg/mL).

Only the hexanic extract showed antifungal activity in all the fungal strains. C. *neoformans* was the strain more sensitive to the hexanic partition effect (MIC=125µg/mL). Figure 1 show the effect of the hexanic partition (in the survival curve) on a fungal strain (C. *neoformans*). Minimum inhibitory concentrations (MIC) had a fungistatic effect on the microbial population, while the minimum fungicidal concentrations (MFC) had a lethal effect on the fungal strain within the first 24 hours. Phytochemical analysis revealed that the hexanic partition containg licosides, terpenes, and flavonoids.

Only the hexanic partition of *C. melanadenia* presented antibacterial activity against eight Gram positive and nine Gram negative bacteria. It was observed that *E. feacalis, S. pneumoniae*, *S. epidermidis* and *E. aerogenes* had the lowest MIC values. In general Gram positive bacteria were more sensitive than the Gram negative ones. In other species of the genus like *C. mimosoides* (Chanwitheesuk *et al*., 2005), *C. tintoria* (López *et al*., 1998; 2008), *C.sappan* (Pawar *et al*., 2008), *C. paraguariensis* (Vattuone *et al*., 2008; Corzo *et al*., 2010) and *C.crista* (Santnami and Yadava, 2011) antimicrobial activity has been reported. This is the first report of a species of the genus that has activity against the strain of *S.pneumoniae*. *S. pneumoniae* infections have resulted in significant morbidity and mortality worldwide in children and adults. It is one of the leading causes of infectious disease including pneumonia, meningitis, bacteremia and otitis media (Deng *et al*., 2013). *C. melanadenia* may be an alternative for the treatment of diseases caused by this bacterial strain.

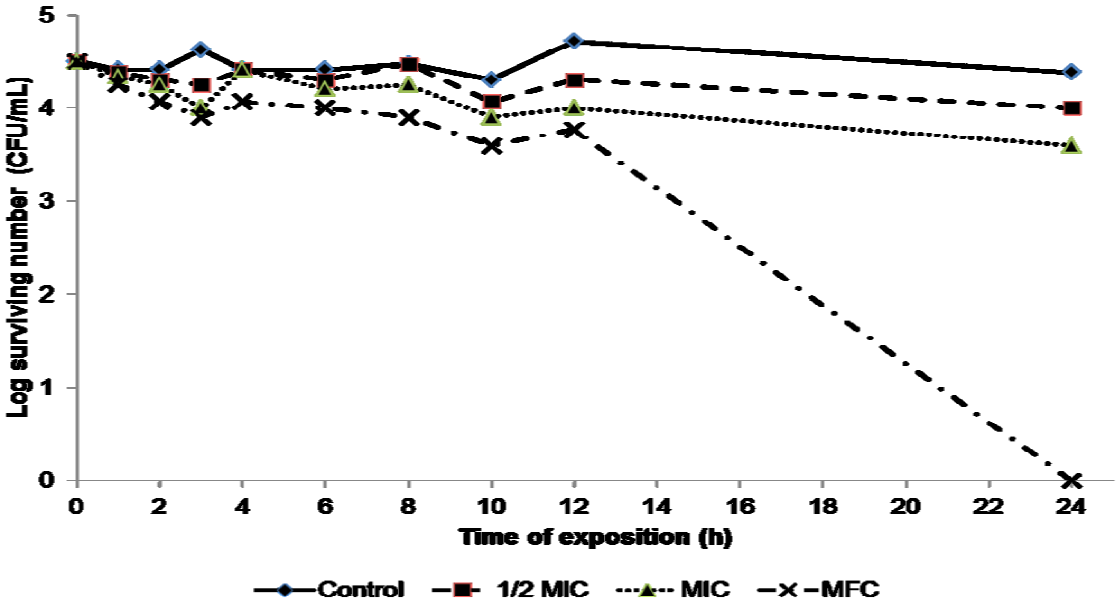
The hexanic partition showed antifungal activity in all the fungal strains. It was observed that *C. neoformans* was the strain more sensitive to the hexanic partition effect. Parekh and Chanda, 2008 reported

Table.1 Antimicrobial activity of *Caesalpinia melanadenia*

Organism	Positive controls			Hexanic partition	
	Inhibition zone (mm)		MIC (mg/mL)		
	Chloramphenicol (25 µg/mL)	Nystatin (30 µg/mL)		Inhibition zone (mm) 2000 µg /disc	MIC (µg /mL)
E. feacalis ATCC 29212	21.67 ± 1.70	---	8	16.00 ± 0.00	60
B. subtilis FES C	16.00 ± 0.47	---	2	14.30 ± 1.15	500
B. subtilis FES I	29.33 ± 2.62	---	2	14.00 ± 1.00	500
S. epidermidis FES I	6.66 ± 1.15	---	2	10.30 ± 0.57	250
S. epidermidis FES C	7.00 ± 1.00	---	2	17.00 ± 1.00	250
S. aureus ATCC 12398	28.00 ± 1.63	---	1	12.30 ± 1.15	750
S.aureus ATCC 29213	27.60 ± 0.11	---	8	15.60 ± 0.57	750
S. pneumonia HA	8.33 ± 0.60	---	16	10.00 ± 0.00	60
V cholera Tor	6.66 ± 0.60	--	1	19.60 ± 0.57	750
V. choleraea gua	10.00 ± 1.00	---	1	16.60 ± 0.57	750
V. cholera cc	27.67 ± 0.47	---	1	16.60 ± 0.57	500
P. aeruginosa ATCC 27853	7.33 ± 0.60	---	8	18.30 ± 0.57	500
E. aerogenes FES C	12.00 ± 0.47	---	4	9.30 ± 0.57	750
E. aerogenes FES I	19.33 ± 0.47	---	4	16.30 ± 0.57	250
S. typhy ATCC 94430	25.67 ± 0.47	---	2	8.30 ± 0.57	750
Y. enterocolitica HA	25.67 ± 0.47	---	4	9.30 ± 0.57	750
E. coli ATCC 25922	21.67 ± 0.47	---	4	na	nd
C. tropicalis HA	---	9.00 ± 1.00	8	25.0 ± 0.00	500
C. albicans FES C	---	9.00 ± 1.00	11	22.3 ± 0.47	500
C. neoformans FES C	---	8.67 ± 0.58	4	22.0 ± 1.73	125
C. tropicalis FES C	---	9.00 ± 1.00	8	25.0 ± 0.00	1000
C. albicans ATCC 10231	---	9.67 ± 0.58	4	26.0 ± 1.00	750
C. glabrata HA	---	7.67 ± 0.58	8	28.0 ± 0.00	750
C. albicans HA	---	9.33 ± 0.58	11	26.0 ± 0.00	500
C. albicans ATCC 14065	---	11.83 ± 2.02	11	26.0 ± 0.00	500
C. albicans FES I	---	9.33 ± 0.58	11	23.3 ± 0.57	500

FES C = strains donated by FES-Cuautitlán, FES I= strains donated by the Clinical Analysis Laboratory of University Hospital Campus Iztacala, HA= strains isolated from a clinical case donated by Hospital Angeles (Metropolitano).na= no activity, nd= no determinated.

Figure.1 Survival curve of *C. neoformans* exposed to hexanic extract of *C. melanadenia.* The hexanic extract was added to each experimental culture in zero time. The concentrations used were: 62.5 µg/mL (½ MIC), 125 µg/mL (MIC), 250 µg/mL (MFC). The control tube did not contain methanol extract.



that *C. pulcherrima* present antifungal activity. As can be seen our results agree with those reported for other species of the genus.

These results showed that the hexanic partition has potential antimicrobial effects against representative human pathogenic bacteria and fungi, such as *E. feacalis, S. pneumonia, S. epidermidis* and *E. aerogenes*, *Cryptococcus neoformans*, and *Candida albicans.* The broad spectrum antibacterial activity exhibited by the hexanic partition of *C. melanadenia* could be linked to its use for respiratory, gastrointestinal and dermatological infection of bacterial and fungal origin in traditional medicine.

Our phytochemical analysis revealed that the hexanic partition containg licosides, terpenes, and flavonoids. These groups of metabolites correspond with that described for the genus, including the predominant phenolic derivatives and terpenes like triterpenoids and diterpenes (Zanin *et al*., 2012). The structural characterization of these compounds by further analysis may promote the drug discovery from plant-based formulations to control the infectious drug-resistant pathogenic microorganisms.

The present study has validated the use of *C. melanadenia* in folk medicine for the treatment of gastrointestinal, respiratory and dermatological diseases. It is hence recommended that further studies in the isolation of active components in the aerial part of the plant should be performed.

Acknowledgement

This research could not have been done without the cooperation of many people in the village of San Rafael Coxcatlan,

Puebla.This research has been supported by project useful San Rafael Coxcatlán Plants (MGU/Useful Plants Project México) Royal Botanical Gardens Kew. UNAM-PAPCA 2011-2012 (11). The authors are grateful to Héctor Cervantes Maya for their technical assistance.

References

Ata, G. E., and Samarasekera, R. 2009. Bioactive chemical constituents of *Caesalpinia bonduc* (Fabaceae). Phytochem. Lett. 2: 106–109.

Argueta, V. A., and Cano, A. J. 1994. Atlas de las Plantas de la Medicina Tradicional Mexicana. Instituto Nacional Indigenista, México.

Christoph, F., P.M. Kaulfers and Sthal-Biskup, E. 2000. A comparative study of the *in vitro* antimicrobial activity of tea tree oils with special reference to the activity of β-triketones. Planta Medica. 66:556-560.

Carvalho, J., J. Teixeira, J.C. Pergentino, C. Souza, K. Jairo, D. Bastos, D. Dos Santos and Sarti, S. 1996. Preliminary studies of analgesic and anti-inflammatory properties of *Caesalpinia ferrea* crude extract. J. Ethnopharmacol. 53: 175-178.

Chakraborthy, G., S. Rohan and Chaitanya, R. 2009. Analgesic activity of chloroform extract of *Caesalpinia pulcherrima*. J. Pharm. Res. 2: 1199-1200.

Chanwitheesuk, A., A. Teerawutgulrag, J. Kilburn and Rakariyatham, N. 2005. Antimicrobial gallic acid from *Caesalpinia mimosoides Lamk*. Food Chem. 100: 1044–1048.

Corzo, A. G., M.A. Sgariglia, M. A. Vattuone, A. Chifarelli, C.A., Zurita and Coronel, F. P. 2010. Extracto alcohólico de hojas de *Caesalpinia paraguariensis* (D. Parodi) Burk. como fuente de principios antimicrobianos contra bacterias patógenas humanas y fitopatógenas. Ciencias Forestales. 18 (1,2): 79-89.

Das, B., Y. Srinivas, S. Sudhakar, I. Mahender, K. Laxminarayana, P. Raghavendar, T. Raju, N. Jakka and Rao, J. 2010. New diterpenoids from *Caesalpinia* species and their cytotoxic activity. Bioorgan. Medicinal Chem. Lett. 20: 2847–2850.

Dávila, P., M. Arizmendi, C. del, A. Valiente-Banuet, J.L. Villaseñor, A.Casas and Lira, R. 2002. Biological diversity in the Tehuacan- Cuicatlán Valley, México. Biodiversidad y Conservacion. 11: 421-442.

Deng, X., D. Church, O.G. Vanderkooi, D.E. Low and Pillai, D. R. 2013. *Streptococcus pneumoniae* infection: a Canadian perspective. Expert Rev. Anti-Infective Therapy. 11:781-91.

Gan, R., X. Xu, L. Song and Li, L. 2010. Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases. Academic J. 4: 2438-2444.

Koneman, W. E., 1991. Diagnóstico microbiológico. Editorial Médica Panamericana, México. 461 pp.

Lennette, H. E., A. Balows, J.W. Hauster and Shadomy, H. J. 1987. Manual de microbiología clínica. 4 edition. Médica Panamericana. Argentina.

López, F. C., V. Garró and Yrei, V. 1998. Acción antimicrobiana de *Caesalpinia tintoria* (Molina) Kuntze o Tara, de diferentes regiones del Perú. Ciencia e Investigación. 5 (1): 28-37.

López, G. M., and Rodríguez, P. M. 2008. Determinación de los componentes fitoquimicos en hoja y semilla de la planta *Caesalpinia bonduc.* Spectro Q. 10: 1.

Pawar, Ch. R., A.D. Landge and Surana,

S. J. 2008. Phytochemical and Pharmacological Aspects of *Caesalpinia sappan.* J. Pharm. Res. 1: 131-138.

Parekh, J., and Chanda, S. 2008. In vitro antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. African. J. Biotechnol. 7: 4349-4353.

Sampietro, D., M. Sgariglia, J. Soberón, E. Quiroga and Vattuone, M. 2009. Colorimetric reactions. In: Sampietro D, Catalán C. Vattuone M (Eds) Isolation, identification and characterization of allele chemicals/Natural Products. USA. Science Publishers.

Santnami, D., and Yadava, R. 2011. Potential Phytochemical from *Caesalpinia crista* Linn. Res. J. Phytochem. 5:22-31.

Srinivas, K., Y. Koteswara, I. Mahender, B. Das, K. Rama, H. Kishore and Murty, U. 2003. Flavanoids from *Caesalpinia pulcherrima*. Phytochem. 63: 789–793.

Srinivasan, Y., S. Sudhakar, B. Das, I. Mahender, K. Laxminarayana, P. Raghavendar, T. Raju, N. Jakka and Rao, J. 2010. New diterpenoids from *Caesalpinia species* and their cytotoxic activity. Bioorgan. Medicinal Chem. Lett. 20: 2847–285.

Vanden Berghe, D. A., and Vlietinck, A. J. 1991. Screening methods for antibacterial agents from higher plants. In Dey, P.M., Harborne, J.B., Hostettman, K. (Eds.), Methods in plant Biochemistry, Assay for Bioactivity. Vol. 6. Academic Press, London. pp 47 - 69.

Vattuone, M. A., A. Martínez and Corzo, G. 2008. Actividad antibacteriana de extractos de hojas de *Caesalpinia paraguariensis*, Par. Burk, "Guayacán". Mole. Medicinal Chem. 15: 37-41.

Wagner, H. 2001. Plant drug analyses. Second Edition, Edit. Springer: Germany. pp. 15-29.

Yodsaoue, O., K. Chatchanok, C.H. Ponglimanont, S. Tewtrakul and Chantrapromma, S. 2011. Potential anti-inflammatory diterpenoids from the roots of *Caesalpinia mimosoides* Lamk. Phytochem. 71: 1756–1764.

Zanin, J. L., B.A. de Carvalho, P.S. Martineli, M.H. dos Santos, J. H. Lago, P. Sartorelli, C. Viegas and Soares, M. G. 2012. The genus *Caesalpinia* L. (Caesalpiniaceae): phytochemical and pharmacological characteristics. Molecul. 17:7887-902.