Effect of Tamarindus indica L. and Manihot esculenta Extracts on Antibiotic-resistant Bacteria

Zenon Machado Lima¹, Lenilson Santos da Trindade¹, Genelane Cruz Santana², Francine Ferreira Padilha¹, Marcelo da Costa Mendonça¹, Luiz Pereira da Costa¹,³, Jorge A. López¹, Maria Lucia Hernández Macedo¹

¹Program in Industrial Biotechnology- Tiradentes University/ Institute of Technology and Research, ²Material Science and Engineering- Federal University of Sergipe, ³Biomaterials and Nanotechnology Laboratory- Technological Institute and Research of the Sergipe State, Aracaju-SE, Brazil

ABSTRACT

Background: The chemical composition of plants used in traditional medicine exhibits biologically active compounds, such as tannins, flavonoids, and alkaloids and becomes a promising approach to treat microbial infections, mainly with drug-resistant bacteria. Objective: The aim of the present study was to evaluate the hydroethanolic leaf extracts of Tamarindus indica (tamarind) and Manihot esculenta (cassava) as antimicrobial potential against Pseudomonas aeruginosa clinical isolated and Methicillin-resistant Staphylococcus aureus. Materials and Methods: Hydroethanolic leaf extracts were prepared and characterized by high-performance liquid chromatography/diode array detection, Fourier transform infrared, 1,1-diphenyl-2-picrylhydrazyl, and ultraviolet-visible methods. The antimicrobial activity against four strains of clinical relevance was evaluated by the microdilution method at minimum inhibitory concentrations. Results: Phenolic compounds such as flavonoids were detected in the plant extracts. T. indica extract at 500 µg/mL showed antimicrobial activity against S. aureus and P. aeruginosa; however, M. esculenta showed only activity against P. aeruginosa in this concentration. Conclusions: Our results suggested that polyphenols and flavonoids present in T. indica leaf extracts are a potential source of antimicrobial compound. The T. indica extract showed antibacterial activity against S. aureus and P. aeruginosa while M. esculenta had effect only on P. aeruginosa meropenem resistant. Key words: Antibiotic-resistant bacteria, antimicrobial, plant extract

SUMMARY

• Antimicrobial effect of T. indica and M. esculenta leaf extract was evaluated.
• T. indica extract displayed activity against S. aureus and P. aeruginosa strains.
• M. esculenta showed effect on P. aeruginosa meropenem resistant.

Abbreviations Used: BHI: Agar brain heart infusion, CAPES: Coordination for the improvement of higher education personnel, DPPH: 1,1-diphenyl-2-picrylhydrazyl, FAPTEC/SE: Foundation for support to research and technological innovation of the state of Sergipe, FTIR: Fourier transform infrared spectroscopy, HPLC: High-performance liquid chromatography, KBr: Potassium bromide, MIC: Minimum inhibitory concentration, MRSA: Methicillin-resistant Staphylococcus aureus, RSC: Radical scavenging capacity, UV‑vis: Ultraviolet-visible.

INTRODUCTION

Ethnobotanical and experimental evidence supports the use of plants due to their wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found to have antimicrobial activity,[1‑4] displaying even synergistic effects with existing antimicrobial drugs.[5] Thus, due to the growing problem of antibiotic resistance of Pseudomonas aeruginosa and Staphylococcus aureus, there is a great interest in plants considering their phytochemicals as potential therapeutic agents.[6‑9] In this context, Tamarindus indica L. and Manihot esculenta have significant importance in traditional medicine for different medicinal purposes.[10‑12] The T. indica L. and M. esculenta phytochemical studies have correlated their antimicrobial activities to several metabolites. T. indica leaf extract revealed the presence of many compounds such as ascorbic acid, β-carotene, polyphenols, and flavonoids (e.g., apigenin, catechin, epicatechin, and naringenin). [13]

Regarding M. esculenta leaf phytocomposition, it indicated the flavonoids, tannins, ascorbic acid, alkaloids, and saponins presence,[14] which display antimicrobial, antioxidant, and anti-inflammatory effects.[15] Overall, phenolic compounds display a structural diversity which could be used for therapeutic intervention due to their biological broad spectrum.[16,17] These bioactivities are attributed to polyphenol

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Lima ZM, da Trindade LS, Santana GC, Padilha FF, da Costa Mendonça M, da Costa LP, Lópeza J, Macedo ML. Effect of Tamarindus indica L. and Manihot esculenta extracts on antibiotic-resistant bacteria. Phcog Res 2017;9:195-9.
interactions with proteins, lipids, and carbohydrates. These interaction induced cell permeability, causing membrane disruption.[14]

Therefore, the chemical composition of T. indica L. and M. esculenta leaf extracts was evaluated, as well as their antimicrobial activity against P. aeruginosa clinical isolated and Methicillin-resistant S. aureus (MRSA).

**MATERIALS AND METHODS**

Plant material and leaf extract preparation

T. indica L. and M. esculenta were collected in Porto do Folha, Sergipe, Brazil. The plants species were identified by Prof. Marla Ibrahim Uehbe de Oliveira and deposited in the Tiradentes University herbarium as AJU154 and AJU155, respectively. The leaves were previously oven-dried at 65°C for 2 days and powdered before extract preparation according to Sultana et al.[19] In brief, 10 g of each dried leaves was mixed with 100 mL of hydro-alcohol (20:80) and ultra-sounded for 1 h. Then, the obtained aqueous phases were concentrated and lyophilized. The dried crude concentrated extracts were stored at −4°C, until used for analyses.

**Determination of total phenolic compounds**

Total phenolic content was determined by Folin-Ciocalteu method.[20] The absorbance was measured in triplicate at 745 nm (DR 5000” ultraviolet-visible [UV-vis] Spectrophotometer) and results expressed as mg of gallic acid equivalents per 100 mg of extract.

**Scavenging activity of 1,1-diphenyl-2-picrylhydrazyl radicals**

The extract for antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Experiments were carried out according to Shaker et al.[21] with some modification. Briefly, 3 mL of each hydroethanolic extract (50–250 µg/mL) was mixed with 750 µL of ethanol solution of DPPH 400 mM. The reaction mixture was shaken thoroughly and incubated in the dark at room temperature for 15 min. The absorbance was measured in triplicate at 517 nm (DR 5000” UV-vis Spectrophotometer). The free radicals percentage inhibition was calculated using the following formula: % RSC = (A0 − A)/A × 100, where RSC = radial scavenging capacity (%), A0 = absorbance of control sample (t = 0 h), and A = absorbance of a tested sample at the end of the reaction (t = 1 h).

**Qualitative phytochemical characterization**

High-performance liquid chromatography/diode array detection

The hydroethanolic leaf extract composition was performed by reversed phase high performance liquid chromatography according to Aznar et al.[22] Dry leaves extract samples (5 mg/mL) were dissolved in methanol and 9 µL was injected into the HPLC (Varian ProStar HPLC system, Walnut Creek, CA). The samples were eluted using 0.1% formic acid and acetonitrile as mobile phase for gradient elution (flow rate = 1 mL/min) through the column Phenomenex C18 (4.6 mm × 100 mm, 2.6 µ particle size, Torrance, CA, USA). The peaks were detected at 280 nm. The compounds identification was done by comparison of their retention time and UV absorption spectrum with those of the standards.

Ultraviolet-visible and fourier transform infrared

To detect T. indica and M. esculenta phenolic compounds, the plant extracts were scanned by UV-vis in the wavelength ranging from 200 to 800 nm using a LAMBDA™ 45 UV-vis Perkin Elmer spectrophotometer. Fourier transform infrared (FTIR) analysis was performed using 10 mg of dried powder of each plant extracts, encapsulated in 100 mg of KBr, and loaded in FTIR spectroscope (Spectrum BX, Perkin Elmer), with a scan range from 400 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹.

**Microorganisms**

Microorganism strains (P. aeruginosa ATCC 27853, S. aureus [MRSA] ATCC 25923, S. aureus [MRSA] ATCC 35591, and S. aureus [MRSA] ATCC 43300) were supplied by the National Institute for Quality Control in Health (INCQS) from the Oswaldo Cruz Foundation (FIOCRUZ). The bacteria maintenance was carried out on Agar Brain Heart Infusion (BHI) (stationary culture) for 37°C at 24 h.

**Minimum inhibitory concentration Determination**

The microdilution method was used to estimate the minimum inhibitory concentration (MIC). Briefly, 96-well microplate was coated with 100 µL BHI, 100 µL extracts leaf (0, 1 g/mL), and 20 µL of microorganisms suspensions previously diluted at 0.5 McFarland turbidity standard. The microplates were incubated at 37°C for 24 h and microbial growth inhibition was determined at 595 nm (Microplate Reader Model 550, Bio Rad, USA). Vancomycin and meropenem were used as standard positive drug against S. aureus and P. aeruginosa, respectively. Experiments were done in triplicate.

**Statistical analysis**

All experimental results were centered using three parallel measurements of the mean ± standard deviation. Analysis of variance was performed followed by Tukey's test as applicable, using the statistical software R (R Development Core Team, 2008). P < 0.05 was considered statistically significant.

**RESULTS**

Phenolic content and antioxidant activity of the extracts

The total phenolic content of T. indica and M. esculenta extracts showed 132.85 ± 1.43 µg/100 mg and 121.64 ± 1.71 µg/100 mg of phenolic compounds, respectively, expressed as gallic acid equivalents.

The RSC evaluated by DPPH method is presented in Figure 1. Both extracts were able to reduce the DPPH in a concentration-dependent manner; however, T. indica showed better antioxidant capacity than M. esculenta compared to silymarin control, ranged from 18% to 75% of RSC.

**Qualitative phytochemical composition**

The chromatographic profiles of M. esculenta leaf extract showed two major peaks whose absorption spectrum matching to 353 nm (band I) and 256 nm (band II) and for T. indica 212 and 269 nm [Figures 2 and 3].
Ultraviolet-visible and Fourier transform infrared analysis

With respect to UV-vis analyses, the extracts showed bands at 283 and 327–328 nm for *T. indica*, whereas bands at 286 and 309–321 nm were observed for *M. esculenta* extract [Figure 4].

On the other hand, the FTIR spectra obtained from *T. indica* and *M. esculenta* extracts showed bands between 3.500 and 3.000 cm⁻¹ assigned to OH group stretching vibration (alcohol and water), and also bands ranged from 2.950 to 2800 cm⁻¹ indicating CH groups vibrations (methyl and methylene) were observed. The peak obtained at 1.680–1.630 cm⁻¹ showed stretching vibration of the C=O (carbonyl) groups while bands between 1.150–1.050 cm⁻¹ and 900–1.300 cm⁻¹ corresponding to C-OH and CH groups, respectively [Figure 5].

Antimicrobial activity

The antibacterial activity of the plant extracts is shown in Table 1. The results indicated that extracts showed antibacterial activities at variable degrees against clinical isolates (*P. aeruginosa*) and MRSA bacteria, with MICs values varying from 125 to 1000 μg/mL. *T. indica* extracts displayed the most important spectrum of activity at 500 and 1000 μg/mL, by inhibiting 100% growth of all microorganisms, whereas *M. esculenta* at 500 μg/mL had satisfactory antibacterial activity only against *P. aeruginosa*. Vancomycin at 1.95 μg/mL inhibited the *S. aureus* growth while meropenem had no activity against *P. aeruginosa* at 500 μg/mL (data not shown).

DISCUSSION

Total phenolic content in plant extracts and their antioxidant activity are strongly related. Phenols have ability of eliminating free radicals due to its hydroxyl groups; therefore, the presence of these compounds in plants may directly contribute to their antioxidant and antimicrobial actions. In this study, the phenolic content of *T. indica* leaf extract was high than in *M. esculenta*. Although the presence of polyphenols in *M. esculenta* was observed, its antioxidant activity is decreased due to glycosides substitutions in their molecular structure, interfering...
Table 1: Minimal inhibitory concentration of Tamarindus indica and Manihot esculenta extract against Pseudomonas aeruginosa and Staphylococcus aureus.

| Leaves extract Concentration (µg/mL) | MIC mean±SD (%) |
|--------------------------------------|-----------------|
|                                      | S. aureus 43300 | S. aureus 25923 | S. aureus 33591 | P. aeruginosa 27853 |
| T. indica                           | T. indica       | T. indica       | T. indica       | T. indica       |
| 125                                  | 17              | 15              | 16              | 24              |
| 250                                  | 18              | 17              | 22              | 21              |
| 500                                  | 100             | 23              | 20              | 100             |
| 1000                                 | 100             | 30              | 27              | 100             |

ZENON MACHADO LIMA, et al.: Leaf Extract Against Resistant Bacteria

Figure 4: Ultraviolet-visible spectrum of Tamarindus indica and Manihot esculenta leaf extract

Figure 5: Fourier transform infrared of Tamarindus indica and Manihot esculenta leaf extract

in this activity.[23] This may explain the lower value of the DPPH with Manihot esculenta extract despite its phenols content. Similar results were obtained by Rahiman et al.[24] by analyzing the antioxidant activity and total phenolic content of plants used as home remedies.

Thus, the better antioxidant capacity of T. indica extract can be explained by a variety of flavonoids and phenolic compounds presence, which act as reducing agents, stabilizing the free radical DPPH.[25]

The extract UV-vis spectra revealed two bands at 310–350 and 250–290 nm, indicating the presence of flavones and flavonols, respectively.[26,32] This result is relevant considering the antimicrobial activity of these compounds.

UV-vis and FTIR data are supported by chromatographic profiles of M. esculenta and T. indica leaf extracts that indicated the phenolic compounds and flavonoids presence, suggested by two typical bands of flavonoids structure and functional groups present (e.g., acids, carbonyl, alcohols, aldehydes, alkanes, and ethers) in these compounds; this is consistent with several data described in scientific literature.[25-32]

According to Jakobek,[19] the detected flavonoids and phenolic compounds in plant extracts act as antimicrobial agents against various human pathogens. The polyphenols mechanism of action on microorganisms is still poorly understood, and some authors suggest that polyphenol acts by reducing the iron availability, inhibiting protein expression, changing the cell membrane permeability, and fluidity.[33-35]

Although antimicrobial compounds present in T. indica leaves are not well elucidated, our results are in line with Cuban and Puerto Rican plant extract studies from the same species, which showed antimicrobial potential probably due to the presence of phenolic compounds.[26,37]

T. indica extract inhibited the S. aureus and P. aeruginosa growth at 500 and 1000 µg/mL. MIC values between 50 and 500 µg/mL indicate strong activity while 600–1500 µg/mL values denote a moderate antimicrobial action,[19] showing that T. indica extract has activity against the bacteria studied.

Although literature reports that Gram-negative bacteria are more resistant than Gram-positive to polyphenols due to the cell wall chemical composition,[29] this resistance was not observed in our results, since the T. indica extract showed an inhibitory capacity against P. aeruginosa.

On the other hand, M. esculenta extract has inhibition at 500 µg/mL only against P. aeruginosa meropenem resistant, evidencing the antimicrobial potential of the extract. The low inhibitory effect of M. esculenta on S. aureus is probably due to attachment of carbohydrates in polyphenols and flavonoids compounds that decreased their antioxidant and antimicrobial activities.[18,31,40]

CONCLUSIONS

In the present study, two plant extracts (T. indica L. and M. esculenta) were studied and their antioxidant and antimicrobial capacities were evaluated. Biomolecules such as polyphenols and flavonoids present in leaf extracts exhibited antimicrobial activity on drug-resistant bacteria. The T. indica extract showed antibacterial effect against Gram-positive and Gram-negative bacteria, while M. esculenta had activity only on P. aeruginosa meropenem resistant. Therefore, results suggest that both extracts are an affordable antioxidants source and have antimicrobial effects.

Acknowledgement

Authors are thankful to CAPES and Fapitec/SE for the financial support and fellowship. We also would like to thank Embrapa Coastal Tablelands, the National Laboratory Síncronton Light and Sergipe Federal University for technical supports.
Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Upadhyay A, Upadhyaya I, Kollanoo-Johny A, Venkatarayanar K. Combating pathogenic microorganisms using plant-derived antimicrobials: A minireview of the mechanistic basis. Biomed Res Int 2014;2014:761741.

2. Singh M, Khatoon S, Singh S, Kumar V, Rawat AK, Mehrotra S. Antimicrobial screening of ethnobotanically important stem bark of medicinal plants. Pharmacognosy Res 2010;2:254-7.

3. Roumy V, Gutiérrez-Choquevica AL, Lopez Mesia JP, Ruiz L, Ruiz Macedo JC, Abedini A, et al. In vitro antimicrobial activity of traditional plant used in mestizo shamanism from the Peruvian amazon in case of infectious diseases. Pharmacogn Mag 2015;11 Suppl 4:S625-33.

4. Kali A. Antibiotics and bioactive natural products in treatment of methicillin resistant Staphylococcus aureus: A brief review. Pharmacogn Rev 2015;9:29-34.

5. Brahim MA, Fadli M, Hassani L, Boulay B, Markouk M, Bekkouche K, et al. Chenopodium ambrosioides var. ambrosioides used in Moroccan traditional medicine can enhance the antimicrobial activity of conventional antibiotics. Ind Crops Prod 2015;71:37-43.

6. Dharmaparakash A, Thandavarayan R, Joseph I, Thomas S. Development of broad-spectrum antibiotic drugs: strategies and challenges. Future Microbiol 2015;10:1035-48.

7. Al-Azzawi A, Alguboori A, Hachim MY, Najat M, Al Shaimaa A, Sad M. Preliminary phytochemical and antibacterial screening of Sesuvium portulacastrum in the United Arab Emirates. Pharmacognosy Res 2012;4:219-24.

8. Dewprashad B, Zakia S, Katayama S, Hendrix R. Antibacterial effects of the extract from Cassia javanica L. on Staphylococcus aureus. J Microbiol 2012;6:19-25.

9. Goyal B, Alok S, Jain SK, Verma A. Evaluation of analgesic activity of ethanolic extract of Sesuvium portulacastrum in the experimental animal model. Int J Pharm Sci Res 2013;4:1984-7.

10. Kuru P. Tamarindus indica and its health related effects. Asian Pac J Trop Biomed 2014;4:676-81.

11. Bhadriya SS, Ganeshpukar A, Narvaria J, Rai G, Jain AP. Tamarindus indica: Extent of explored potential. Pharmacogn Rev 2011;5:73-81.

12. Nwodo UU, Obiyeke GE, Chigor VN, Okoh AI. Assessment of Tamarindus indica extract for antibacterial activity. Int J Mol Sci 2011;12:6385-96.

13. Samina KK, Shakti W, Shahzadi S, Kazi TG, Usmangahi K, Kabir A, et al. Chemical constituents of Tamarindus indica L. medicinal plant in Sindh. Pak J Bot 2008;40:2553-9.

14. Blagbrough IS, Bayoumi SA, Rowan MG, Beeching JR. Cassava: An appraisal for clinical use. Ann Trop Med Parasitol 2008;102:1940-51.

15. Raimi MM, Oyekanmi AM, Farombi AG. Proximate and phytochemical composition of leaves of Ceiba pentandra, Manihot esculentus and Abelmoschus esculentus in Southwestern Nigeria. Sci Res J 2014;2:30-4.

16. Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients 2010;2:1231-46.

17. Asowata-Ayodele AM, Otunola GA, Afolayan AJ. Assessment of the polyphenolic content, free radical scavenging, anti-inflammatory, and antimicrobial activities of aqueous and ethanolic extracts of Lippia javanica (Burm. F.) spreng. Pharmacogn Mag 2016;12:353-62.

18. Jakobek L. Interactions of polyphenols with carbohydrates, lipids and proteins. Food Chem 2015;175:556-67.

19. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 2009;14:2167-80.

20. Slinkard K, Singleton VL. Total phenol analyses: Automation and comparison with manual methods. Am J Enol Vitic 1977;28:49-55.

21. Shaker E, Mahmoud H, Mnaa S. Silymarin, the antioxidant component and Silybum marianum extracts prevent liver damage. Food Chem Toxicol 2010;48:803-6.

22. Aznar O, Checa A, Oliver R, Hernández-Cassou S, Saurina J. Determination of polyphenols in wines by liquid chromatography with UV spectrophotometric detection. J Sep Sci 2011;34:527-35.

23. Manual Clinical and Laboratory Standards Institute (CLSI). National Committee for Clinical Laboratory Standards. Reference Method for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard-Ninth Edition: CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.

24. Mamta, Mehrsutra S, Armitab, Kirar V, Vats P, Nandi SP, et al. Phytochemical and antimicrobial activities of Himalayan Cordyceps sinensis (Berk.) SCC. Indian J Exp Biol 2015;53:36-43.

25. Rekha K, Sivasubramanian C, Thiuvengadam M. Evaluation of polyphenol composition and biological activities of two samples from summer and winter seasons of Ligularia tischanii var. Spiciformis Nakai. Acta Biol Hung 2015;66:179-91.

26. D’Sousa’ Costa CO, Ribeiro PR, Loureiro MB, Simões RC, de Castro RD, Fernández LG. Phytochemical screening, antioxidant and antibacterial activities of extracts prepared from different tissues of Schinus terebinthifolius Raddi that are cultivated in the coast of Bahia, Brazil. Pharmazin Mag 201511;11:807-14.

27. Pochapski MT, Fosquera EC, Esmerino LA, Dos Santos EB, Farago PV, Santos FA, et al. Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves’ extract from Ipomoea batatas (L). Lam. Pharm. Pharmazin Mag 2011;7:165-70.

28. Prawat H, Mahidol C, Churirawat S, Prawat U, Tuntwachuwit-tik P, Tootpaktong U, et al. Cyanogenic and non-cyanogenic glycosides from Manihot esculenta. Phytochemistry 1999;40:1167-73.

29. Rahman S, Tantry BA, Kumar A. Variation of antioxidant activity and phenolic content of some common home remedies with storage time. Afr J Tradit Complement Altern Med 2012;10:124-7.

30. Markham RK, Mitchell EA, Wilkins AL, Daldy JA, Lu Y. HPLC and GC-MS identification of the major organic constituents in New Zealand propolis. Photochemistry 1996;42:205-11.

31. Tsimogiannis D, Samiotaki M, Papayannaki G, Scalbert A, Expert D. Iron withholding by plant polyphenols and naturally occurring antioxidants with different media: A UV-visible spectroscopic study. Spectrochim Acta A Mol Biomol Spectrosc 2010;75:134-16.

32. Daglia M. Polyphenols as antimicrobial agents. Curr Opin Biotechnol 2012;23:174-81.

33. Mila I, Scabert A, Expert D. Iron withholding by plant polyphenols and resistance to pathogens androts. Phytochemistry 1996;42:1551-6.

34. Petti S, Scully C. Polyphenols, oral health and disease: A review. J Dent 2009;37:413-23.

35. Escalona-Arranz JC, Pérez-Roses R, Urdaneta-Laffita I, Camacho-Pozo MI, Rodríguez-Amado J, Licea-Jiménez I. Antimicrobial activity of extracts from Tamarindus indica L. leaves. Pharmazin Mag 2010;6:8-22.

36. Meléndez PA, Capriles VA. Antibacterial properties of tropical plants from the coast of Bahia, Brazil. Pharmazin Mag 2015;11:807-14.

37. Aligiani N, Kalmouzakis E, Mitiku S, Chinou IB. Composition and antimicrobial activity of the essential oils of two Origanum species. J Agric Food Chem 2001;49:4168-70.

38. Nakamura K, Ishiyama K, Sheng H, Ikai H, Kanno T, Niwano Y. Bactericidal activity of the essential oils of two species. J Agric Food Chem 2015;63:7707-13.

39. Abd Aziz SM, Low CN, Chai LC, Abd Razak SS, Selamat J, Son R, et al. Screening of selected Malaysian plants against several food borne pathogen bacteria. Int Food Res J 2011;18:1195-201.

Pharmacognosy Research, Volume 9, Issue 2, April–June, 2017