**Ahmad I, Aqil F, Owais M.**(Eds.) 2006. Modern phytomedicine: turning medical plants into drugs. *Weinheim: Wiley-VCH.*Verlag GmbH & Co. KGaA, Weinheim.

**Ahmed S, Shahid M, Fatima N, Khan F, Tayyaba U.** 2020. Candidemia – Changing trends from *Candida albicans* to non-albicans *Candida* from a tertiary care center in western UP, India. *CHRISMED J Health Res.* **7:** 167-172.

**AkhoundiM, Kuhls K, Cannet A, Votýpka J, Marty P, Delaunay P, Sereno D.** 2016. A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. *PLoSNegl Trop Dis*. **10:** 1-40.

**Albaayit SF, Abba Y, Rasedee A, Abdullah N.** 2015. Effect of *Clausena excavata* Burm. f. (Rutaceae) leaf extract on wound healing and antioxidant activity in rats. *Drug Des Devel Ther.***9:** 3507-3518.

**Albaayit SF, Maharjan R, Abdullah R, Noor MH.** 2021. Anti-*Enterococcus faecalis*, cytotoxicity, phytotoxicity, and anticancer studies on *Clausena excavata* Burum. f. (Rutaceae) leaves. *BioMed Res International*. **2021**: 3123476.

**Ali AM, Ismail NH, Mackeen MM, Yazan LS, Mohamed SM, Ho ASH, Lajis NH.** 2000. Antiviral, cyototoxic and antimicrobial activities of anthraquinones isolated from the roots of *Morinda elliptica*. *Pharm Biol*. **38:** 298-301.

**Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahuand A, Bora U**. 2008. Indian medicinal herbs as source of antioxidants. *Food Res Int*. **41:** 1-15.

**Al-Tawfiq JA, Alhumaid S, Alshukairi AN, Temsah M-H, Barry M, Al Mutair A**, et al. 2021. COVID-19 and mucormycosis super infection: the perfect storm. *Infection*. **49**: 833–853.

**Alvar J, Aparicio P, Aseffa A, Boer MD, Cañavate C, Dedet JP, Gradoni L, Horst RT, López-Vélez R, Moreno J.** 2008. The relationship between Leishmaniasis and AIDS: the Second 10 Years. *Clin Microbiol Rev*. **21:** 334-359.

**Alviano DS, Alviano CS.** 2009. Plant extracts: search for new alternatives to treat microbial diseases. *J Plant Bioinform Biotech*. **10:** 106–121.

**Amin A, Vartanian A, Poladian N, Voloshko A, Yegiazaryan A, Al-Kassir AL, Venketaraman V.** 2021. Root Causes of Fungal Coinfections in COVID-19 Infected Patients. *Infect Dis Rep*. **13:** 1018–1035.

**Amnzadeh Y, Izaddoost M, Soltanpoor A, Mahami-Oskouei M, Taheri M, Kalantari M, Kalantari N, Taran, M Ebrahimi S.** 2006. Inhibit effect of *Allium hirtifolium* Boiss. (Persian shallot) hydroalcoholic extract on the growth of *Leishmania infantum in vitro*. *J Med Plant Res*. **4:** 48-52.

**Antolovich M, Prenzler PD, Patsalides E, McDonald S, and Robards K**. 2002. Methods for testing antioxidant activity. *The Analyst*. **127**: 183-198.

**Aranjani JM, Manuel A, Abdul Razack HI, Mathew ST**2021. COVID-19–associated mucormycosis: Evidence-based critical review of an emerging infection burden during the pandemic's second wave in India. *PLoS Negl Trop Dis.***15**: e0009921.

**Araújo MGF, Pacífico M, Vilegas W.** 2013. Evaluation of *Syngonanthusnitens* (Bong.) Ruhl. extract as antifungal and in treatment of vulvovaginal candidiasis. *Med Mycol*.**51:** 673–682.

**Arbab IA, Abdul AB, Aspollah M, Abdelwahab SI, Ibrahim MY, Ali Z.** 2012. A review of traditional uses, phytochemical and pharmacological aspects of selected members of *Clausena* genus (Rutaceae). *J Med Plants Res*. **6**: 5107-18.

**Armstrong-James D, Meintjes G, Brown GD.** 2014. A neglected epidemic: fungal infections in HIV/AIDS. *Trends Microbiol*.**22:** 120-127.

**Arraché Gonçalves G, Eifler-Lima VL, von Poser GL.** 2021. Revisiting nature: a review of iridoids as a potential antileishmanial class. *Phytochem Rev*. **1:** 1-26.

**Asem BD, Laitonjam WS.** 2008. Isolation and antimicrobial studies of the compounds isolated from the stem bark of *Ficus hispida*Linn. *Asian J Chem*. **20**: 6027–6032.

**Ashford RW.** 1997. The leishmaniasis as model zoonoses. *Ann Trop Med Parasitol*. **91**: 693-701.

**Azmi MB, Qureshi SA.** 2013. *Rauwolfia serpentina* ameliorates hyperglycemic, haematinic and antioxidant status in alloxan-induced diabetic mice. *J Appl Pharm Sci*.**3:** 136-141.

**Badiee P, Hashemizadeh Z.** 2014. Opportunistic invasive fungal infections: diagnosis & clinical management.*Indian J Med Res*. **139**: 195–204.

**Badirzadeh A, Heidari-Kharaji M, Fallah-Omrani V, Dabiri H, Araghi A, Chirani AS.** 2020. Antileishmanial activity of *Urtica dioica* extract against zoonotic cutaneous leishmaniasis. *PLoSNegl Trop Dis*. **14:** 1-20.

**Bavaresco L.** 2003. Role of viticultural factors on stilbene concentrations of grapes and wine. *Drugs Exp Clin Res*. **29**: 181–187.

**Ben Ami R, Lewis RE, Kontoyiannis DP.** 2009. Invasive mould infections in the setting of hematopoietic cell transplantation: current trends and new challenges. *CurrOpin Infect Dis*. **22:** 376–384.

**Bernuci KZ, Iwanaga CC, Fernadez-Andrade CMM, Lorenzetti FB, Torres-Santos EC, Faiões VD, Gonçalves JE, do Amaral W, Deschamps C, Scodro RB, Cardoso RF, Baldin VP, Cortez DA**. 2016. Evaluation of chemical composition and antileishmanial and antituberculosis activities of essential oils of *Piper* species. *Molecules*. **21:** 1698.

**Bhattacharyya UC.** 1997. Flora of West Bengal Vol. I. Botanical Survey of India, Kolkata.1–78.

**Bhuyan SI, Meyiwapangla, Laskar I.** 2014. Indigenous Knowledge and traditional use of medicinal plants by four major tribes of Nagaland, North East India. *Int J Innov Res Sci Eng Technol*.**1:** 481-484.

**Bora A, Devi P, Borthakur SK.** 2012. Phyto remedies of jaundice, a traditional approach on Majuli, special reference to Satra culture people, Assam. *Asian J Plant Sci Res*. **2:** 664-669.

**Borthakur SK, Choudhury BT, Gogoi R.** 2004. Folklore hepato-protective herbal recipes from Assam in Northeast India.*Ethnobot*. **16:** 76-82.

**Bose S**. 1952. Rauwolfinine, the new alkaloid of the root of *Rauwolfia serpentine* Benth. *J Indian Chem Soc*.**1:** 47.

**Bose S.** 1954. Rauwolfinine: A new alkaloid of *Rauwolfia serpentine* Benth. II. Studies on the ultraviolet and infrared absorption spectra. *J Indian Chem Soc*. **31:** 311-318.

**Braconier JH, Miomer H.** 1993. Recurrent episodes of thrombocytopenia during treatment with sodium stibogluconate.*J Antimicro Chemother*. **31**: 187-188.

**Brendle JJ, Outlaw A, Kumar A, Boykin DW, Patrick DA, Tidwell RR.** 2002. Anti-Leishmanial activities of several classes of aromatic dications. *Antimicrob Agents Chemother*. **46**: 797-807.

**BriedbachT,Scory S, Krauth-Siegel RL, Steverding D.** 2002. Growth inhibition of bloodstream forms of *Trypanosoma brucei* by the iron chelator deferoxamine. *Int J Parasitol*.**32:** 473-479.

**Brown JE, Rice-Evans CA**. 1998. Luteolin rich artichoke extract protects low density lipoprotein from oxidation *in vitro*. *Free Radical Res*. **29:** 247-255.

**Bunkar AR.** 2017. Therapeutic uses of *Rauwolfia serpentina*. *Int J Adv Sci Res.***2:**23-26.

**Burki lll H**. 1935. Adictionary of the economic products of the Malay Peninsula. Ministry of agriculture (Malaysia). *Crown Agents*. London. **2:** 2444.

**Bushra S, Anwar F.** 2008. Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. *Food Chem*. **108:** 879–884.

**Casadevall A, Kontoyiannis DP, Robert V.** 2019. On the emergence of *Candida auris*: climate change, azoles, Swamps, and Birds. *mBio*.**10:** 1397-19.

**Ceole LF, Cardoso MDG, Soares MJ.** 2017. Nerolidol, the main constituent of *Piper aduncum* essential oil, has anti-*Leishmania braziliensis* activity. *Parasitol*. **144:** 1179-1190.

**Chakrabarti A**. 2005. Microbiology of systemic fungal infections. *J Postgrad Med*. **51**: 16-20.

**Chandra J, Anand V, Patwari AK, Mandal RN, Aneza S, Sharma D.** 1995. Kala-azar: experience from a non-endemic area in India. *J Trop Pediatr*. **41**: 298-300.

**Channon JY, Roberts MB, Blackwell JM**. 1984. A study of the differential respiratory burst activity elicited by promastigotes and amastigotes of *Leishmania donovani* in murine resident peritoneal macrophages. *Immunology*. **53**: 345-355.

**Chaudhuri Rai HN, Mollah HA, Pal DC**. 1982. Plants used in traditional medicine by some tribals of Jalpaiguri district, West Bengal. *Bull Botanical Survey India*. **24**: 87-90.

**Chhetri HP, Yogol NS, Sherchan J, KCA Mansoor S, Thapa P.** 2008. Phytochemical and antimicrobial evaluations of some medicinal plants of Nepal. *Kathmandu University Journal of Science, Engineering and Technology*. **1**: 49-54.

**Chiang LY, Ejzykowicz DE, Tian ZQ, Katz L, Filler SG.** 2006. Efficacy of ambruticin analogs in a murine model of invasive pulmonary aspergillosis. *Antimicrob Agents Chemother*. **50**: 3464-3466.

**Chiurlo M., Mastrangelo A., Ripa M., Scarpellini P.**2021. Invasive fungal infections in patients with COVID-19: A review on pathogenesis, epidemiology, clinical features, treatment, and outcomes. *New Microbiol*. **44**: 71–83.

**Choudhary K, Singh M, Pillai U**. 2008. Ethnobotanical survey of Rajasthan an update. *American-Eurasian J Botany*. **1**: 38-45.

**Chuang P, Lee C, Chou J, Murugan M, Shieh B, Chen H.** 2007. Anti-fungal activity of crude extracts and essential oil of *Moringa oleifera* Lam. *Biores Technol*. **98:** 232–236.

**Clancy CJ, Nguyen MH.** 2017. Emergence of *Candida auris*: An international call to arms. *Clin Infect Dis*. **64:** 141–143.

**Cowan MM**. 1999. Plant products as antimicrobial agents. *Clin Microbiol Rev*. **12**: 564-582.

**Cox FE**. 2002. History of human parasitology. *Clin Microbiol Rev*.**15**: 595-612.

**Criagg GM, David JN**. 2001. Natural product drug discovery in the next millennium. *J Pharm Bio*. **39:** 8-17.

**Croft SL, Sundar S, Fairlamb AH**. 2006. Drug resistance in leishmaniasis. *Clin Microbiol Rev*. **19**: 111-126.

**Dalimi A, Arbabi M, Naserifar R.** 2013. The effect of aqueous extraction of *Artemisia siasieberi* Besser and *Scrophularia triata* Boiss. on *Leishmania major* under in vitro conditions. Iran J Med Arom Plant. **29**: 237-246.

**Das AK, Tag H.** 2006. Ethnomedicinal studies of the Khamti tribe of Arunachal Pradesh. *Indian J Tradit Knowl*. **5:** 317-322.

**Das, A. K., Dutta, B. K., & Sharma, G. D.** 2008. Medicinal plants used by different tribes of Cachar district, Assam. *Indian J Tradit Knowl*. **7**: 446-454.

**Das B, Talukdar AD, Choudhury MD.** 2014. A few traditional medicinal plants used as antifertility agents by ethnic people of Tripura, India. *Int J Pharm Pharm Sci*. **6:** 47-53.

**Das SN, Janardhanan KP, Roy SC**. 1983. Some observations on the ethnobotany of the tribes of Totopara and adjoining areas in Jalpaiguri Districts of West Bengal.*J Econ Taxon Bot*. **4:** 453-474.

**Dasbach EJ, Davies GM, Teutsch SM.** 2000. Burden of aspergillosis related hospitalizations in the United States. *Clin Infect Dis*. **31:** 1524–1528.

**Dasgupta B, Roychoudhury K, Ganguly S, Akbar MA, Das P, Roy S.** 2003. Infection of human mononuclear phagocytes and macrophage – like THP1cell with *Leishmania donovani* results in modulation of expression of a subset of chemokines and a chemokine receptor. *Scan J Immunol*. **57**: 366-374.

**Dashtpeima AR, Manzoori L, Arefkhan N, Shahryari S, Mohseni M, Abbasi M.** 2015. The effect(s) of *Matricaria chamomilla* on *Leishmania* major ulcers in Balb/ cMice. Armaghane Danesh. Yasuj Univ Med Sci J. 20: 127-137.

**Davis BJ.** 1964. Disc electrophoresis. 11. Method and application to human serum proteins. *Ann NY Acad Sci.***121:** 404.

**de Paula RC, da Silva SM, Faria KF, Frézard F, Moreira CPS, Foubert K, Lopes JCD, Campana PRV, Rocha MP, Silva AF, Silva CG, Pieters L, Almeida VL.** 2019. *In vitro*antileishmanial activity of leaf and stem extracts of seven Brazilian plant species. *J Ethnopharmacol*. **232:** 155-164.

**Debbarma M, Pala NA, Kumar M, Bussman RW.** 2017. Traditional knowledge of medicinal plants in tribes of Tripura in northeast, India. *Afr J TraditCompl Altern Med*. **14:** 156-168.

**Delgado-Altamirano R, Monzote L, Piñón-Tápanes A, Vibrans H, Rivero-Cruz JF, Ibarra-Alvarado C, Rojas-Molina A**. 2017. *In vitro* antileishmanial activity of Mexican medicinal plants. *Heliyon*. **3:** 1-19.

**Demarchi IG, Terron Mde S, Thomazella MV, Mota CA, Gazim ZC, Cortez DA, Aristides SM, Silveira TG, Lonardoni MV.** 2015. Antileishmanial and immunomodulatory effects of the essential oil from *Tetradenia riparia* (Hochstetter) Codd. *Parasite Immunol*. **38:** 64-77.

**Demirezen S, Donmez HG, Ozcan M, Beksac MS.**2015. Evaluation of the relationship between fungal infection, neutrophil leukocytes and macrophages in cervicovaginal smears: Light microscopic examination. *J Cytol*. **32:** 79–84.

**Denning DW, Hope WW**. 2010. Therapy for fungal diseases: opportunities and priorities. *Trends Microbiol*.**18**: 195-204.

**Dewick PM.**1996. Tumor inhibition from plants. *In Trease and Evans Pharmacognosy,* Elsevier Health Sciences. Philadelphia, PA, USA.

**Dey R, Mitra S, Datta SC**. 1995. Cyclic AMP mediates change in superoxide dismutase activity to monitor host–parasite interaction in *Leishmania donovani*. *J Parasitol*. **81**: 683–686.

**Dhar Purkayastha G, Mangar P, Saha A, Saha D.**2018. Evaluation of the biocontrol efficacy of a *Serratia marcescens* strain indigenous to tea rhizosphere for the management of root rot disease in tea. *Plos One*.**13:**1-27.

**Donovan C.** 1903. On the possibility of the occurrence of trypanosomiasis in India. *Br Med J*. **2**:79.

**Duarte MCT, Figueira GM, Sartoratto A, Rehder VLG, Delarmelina C**. 2005. Anti-*Candida* activity of Brazilian medicinal plants. *J Ethnopharmacol*. **97:** 305-310.

**Dutta BK, Dutta PK.** 2005. Potential of ethnobotanical studies in North East India: An overview. *Indian J TraditKnowl*. **4**: 7-14.

**Dzoyem JP, Tangmouo JG, Lontsi, D, Etoa FX, Lohoue PJ.** 2007. *In vitro* antifungal activity of extract and plumbagin from the stem bark of *Diospyros crassiflora Hiern (Ebenaceae). Phytother Res.***21**: 671-674.

**Edoga HO, Okwu DE, Mbaebie BO.** 2005. Phytochemicals constituents of some Nigerian medicinal plants. *Afr J Biotechnol*. **4**: 685-688.

**Elizabeth KM.**2017. Antimicrobial Activity of *Rauwolfa serpentina*. *J Hum Ecol*. **12:** 375-377.

**Emami SA, Rabe SZT, Ahi A, Mahmoudi M.** 2012. Inhibitory activity of eleven *Artemisia* species from Iran against *Leishmania major* parasites. *IranJ basic Med Sci*. **15:** 807-811.

**Endo EH, Garcia Cortez DA, Ueda-Nakamura T, Nakamura CV, Dias Filho BP.** 2010. Potent antifungal activity of extracts and pure compound isolated from pomegranate peels and synergism with fluconazole against *Candida albicans*. *Res Microbiol*. **161:** 534-540.

**Endreß S, Suda S, Takayama H, Aimi N, Sakai SI, Stöckigt J.** 2007. Isolation, identification, and chemical synthesis of 6α-hydroxyraumacline: A novel alkaloid from cultivated *Rauwolfia serpentina* Cells. *Planta med*. **58:** 410-412.

**Epand RF.** 2007. Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins). *Biochim Biophys Acta*. **1768**: 2500-2509.

**Eslami G, Shams A, Fasahat M.**2013. Effects of S-allyl-cysteine on survival, apoptosis, and proliferation of *Leishmania major in vitro*. *J Isfahan Med Sch*.**31:**121-130.

**Ezatpour B, Dezaki ES, Mahmoudvand H, Azadpour M, Ezzatkhah F. 2015.** In vitro and in vivo antileishmanial effects of *Pistacia khinjuk* against *Leishmania tropica* and *Leishmania major*. *Evid Based Complement Alter Med*. **2**: 1-6

**Ezeigbo II, Ezeja MI, Madubuike KG, Ifenkwe DC, Ukweni IA, Udeh NE, Akomas SC.** 2012. Antidiarrhoeal activity of leaf methanolic extract of *Rauwolfia serpentina*. *Asian Pac J Trop Biomed*. **2:** 430-432.

**Fabri RL, Freitas JC, Lemos AS, Campos LM, Diniz IO, Pinto NC, Silva TP, Palazzi C, Marchesini P, Monteiro C, Barbosa AF.** 2021. Spilanthol as a promising antifungal alkylamide for the treatment of vulvovaginal candidiasis. *Med Mycol*. **59**: 1210-24.

**Fahey JW, Zalcmann AT, Talalay P.** 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochem*. **56**: 5–51.

**Fahey JW**. 2005. *Moringa oleifera*: A Review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. *Phytochem*. **47:** 123-157.

**Faizi S, Siddiqui BS, Saleem R, Aftab K, Shasheen F, Gilani AH**. 1998. Hypotensive constituents from the pods of *Moringa oleifera*. *Planta medica*. **64:**225–228.

**Faizi S, Siddiqui BS.** 1992. Isolation and structure elucidation of novel hypotensive agents, niazinin A, niazinin B, niazimicin and niaziminin A plus B from *Moringa oleifera*: The first naturally occurring thiocarbamates. *J Chem Soc Perkin Trans.***1**:3237–3241.

**Faizi S. Siddiqui BS, Saleem R, Siddiqui S, Aftab K, Gilani AH.** 1995. Fully acetylated carbamate and hypotensive thiocarbamate glycosides from *Moringa oleifera*. *Phytochem*. **38:** 957–963.

**Fattman CL, Scaefer LM, Oury TD.** 2002. Extracellular superoxide dismutase in biology and medicines. *Free Radical Biol Med.***35:** 236-256.

**Firacative C**. 2020. Invasive fungal disease in humans: are we aware of the real impact? *Mem Inst Oswaldo Cruz*. **115:** 1-9.

**Folgueira I, Lamas J, Felipe APD, Sueiro RA, Leiro JM.** 2019. Identifcation and molecular characterization of superoxide dismutase sisolated from ascuticociliate parasite: physiological role in oxidative stress. *Sci Rep.***9:** 13329.

**Foroutan-Rad M, Tappeh KH, and Khademvatan S.** 2017. Antileishmanial and immunomodulatory activity of *Allium sativum* (Garlic): A Review. *Evid Based Complement Alternat Med.* **22:** 141-155.

**Fournet A, Munoz V.** 2002. Natural products as trypanocidal, antileishmanial and antimalarial drugs. *Curr Top Med Chem.***2:** 1215-1237.

**Francis JA, Jayaprakasam B, Olson LK, Nair MG**. 2004. Insulin secretagogues from *Moringa oleifera* with cyclooxygenase enzyme and lipid peroxidation inhibitory activities. *Helvetica Chimica Acta*. **87**: 317–326.

**Fransworth NR**. 1988. Screening plants for new medicines. *Biodiversity*. **15**: 81-99.

**Friedman EI**. 1982. Endolithic microorganisms in the antarctic cold desert. *Science*. **215:** 1045–1053.

**Fuglie LJ.**2001. The Miracle Tree: *Moringa oleifera*: Natural Nutrition for the Tropics. Church World Service, Dakar. 68

**Furuta S, Nishiba Y, Suda I.** 2010. Fluorometric assay for screening antioxidative activities of vegetables. *J Food Sci*. **62**: 526–528.

**Fuzibet JG, Marty P, Taillan B, Bertrand F, Pras P, Pesce A, Le Fichoux Y, Dujardin P.** 1988. Is *Leishmania infantum* an opportunistic parasite in patients with anti-human immunodeficiency virus anti-bodies? *Archs Int Med.* **148:** 1228.

**Garber, G.** 2001. An Overview of Fungal Infections. *Drugs* **61**: 1–12.

**Garrido JC, Salinas-Sandoval M, Díaz-López A, Jácquez-Ríos P, Arriaga-Alba M, Ordaz-Pichardo C. 2015.** *In vitro* and *in vivo* antifungal activity, liver profile test, and mutagenic activity of five plants used in traditional Mexican medicine. *Rev Bras Farmacogn.***25:** 22-28.

**Gazzinelli RT, Wysocka M, Hieny S, Scharton-Kersten T, Cheever A, Kuha R, Muller W, Trinchieri G, Sher A.** 1996. In the absence of endogenous IL-10, mice actually infected with *Toxoplasma gondii* succumb to a lethal immune response dependent on CD4+ T cells and accompanied by overproduction of IL-12, IFN-gamma and TNF-alpha. *J Immunol*.**157**: 798-805.

**Gawade BV, Fegade SA.** 2012. *Rauwolfia* (reserpine) as a potential antihypertensive agent: A review. *Int J Pharm Phytopharmacol Res.* **2:** 46-49.

**Ge G, Li D, Mei H, Lu G, Zheng H, Liu W, Shi D.** 2019. Different toenail onychomycosis due to Rhodotorula mucilaginosa and *Candida parapsilosis* in an immunocompetent young adult. Medical mycology case reports. 24: 69-71.

**Ghosh AK, Bhattacharya FK, Ghosh DK.** 1985. *Leishmania donovani*: amastigote inhibition and mode of action of berberine. *Exp Parasitol*. **60:** 404-413.

**Ghosh S, Goswami S, Adhya S.** 2003. Role of superoxide dismutase in survival of *Leishmania* within the macrophage. *Biochem J.* **369:** 447–452.

**Gilligan PH, Shapiro DS, Miller MB.** 2014. Cases in medical microbiology and infectious diseases. Fourth Edition. ASM Press. Washington D.C.

**Giunchetti RC, Reis AB, da Silveira-Lemos D, Martins-Filho OA, Correa-Oliveira R, Bethony J, et al.** 2008. Antigenicity of a whole parasite vaccine as promising candidate against canine leishmaniasis. *Res Vet Sci.* **85**:106-112.

**Glew RH, Saha AK, Das S, Remaley AT.** 1988. Biochemistry of the *Leishmania* species. *Microbiol Rev.* **52**: 412-432.

**Gnat S, Łagowski D, Nowakiewicz A, Dylazg M**. 2021. A global view on fungal infections in humans and animals: opportunistic infections and microsporidioses. *J Appl Microbiol*. **131:** 2095-2113.

**Gopalan C, Rama S, Balasubramanian SC**. 1989. Nutritive value of Indian foods. National Institute of Nutrition, Indian Council of medical Research. New Delhi.

**Govindarajan R, Vijayakumar M, Pushpangadan P**. 2005. Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. *J Ethnopharmacol*. **99:** 165-178.

**Guntupalli C, Kumar GS, Kumar AS, Tubati T.** 2012. Evaluation of antioxidant activity of the methanolic leaf extract of *Clausena excavata* Burm. f. (Rutaceae*)* using the lipid peroxidation model. *Pharmacognosy Journal*, **4:**22-25.

**Gupta S, Raychaudhuri B, Banerjee S, Das B, Mukhopadhaya S, Datta SC.** 2010. Momordicatin purified from fruits of *Momordica charantia* is effective to act as a potent antileishmania agent. *Parasitol Int*. **59:** 192-197.

**Hagerman AE.** 2002. The Tannin Handbook. Miami University, Ohio, USA.

**Han X, Shen T, Lou H**. 2007. Dietary polyphenols and their biological significance. *Int J Mol Sci*. **8**: 950-988.

**Han X, Lamshöft M, Grobe N, Ren X, Fist AJ, Kutchan TM, Zenk, MH.** 2010. The biosynthesis of papaverine proceeds via (S)-reticuline. *Phytochemistry* 71: 1305–1312.

**Handman E**. 2001. Leishmaniasis: current status of vaccine development. *Clin Microbiol Rev.***14** :229-243.

**Harborne JB, Baxter H, Moss GP**. 1999. Phytochemical dictionary: Handbook of bioactive compounds from plants. 2nd ed. Taylor and Francis, London.221-234

**Harborne JB**. 1973. Phytochemical methods, London. Chapman and Hall, Ltd. 49-188.

**Hart DT, Coombs GH**. 1982. *Leishmania mexicana:* energy metabolism of amastigotes and promastigotes. *Exp Paracitol*. **54**:397-409.

**Hashemi SA, Abediankenari S, Ghasemi M, Azadbakht M, Yousefzadeh Y, Dehpour AA.** 2011. The effect offig tree latex (*Ficus carica*) on stomach cancer line. *Iran Red Crescent Med J*. **13:** 272–275.

**Herwaldt BL, Berman JD.** 1992. Recommendations for treating leishmaniasis with sodium stibogluconate (Pentosam) and review of pertinent clinical studies. *Am J Trop Med Hyg*.**46:** 296-306.

**Hoenigl M, Seidel D, Carvalho A, Rudramurthy SM, Arastehfar A, Gangneux JP, Nasir N, Bonifaz A, Araiza J, Klimko N.** et al. 2022. The emergence of COVID-19 associated mucormycosis: A review of cases from 18 countries. *Lancet Microbe*. 2022. Jan 25.

**Hong M, Li S, Tan HY, Wang N, Tsao SW, Feng Y.** 2015. Current status of herbal medicines in chronic liver disease therapy: The biological effects, molecular targets and future prospects. *Int J Mol Sci*. **16:** 28705-28745.

**Horiuchi N, Nakagava K, Sasaki Y, Minato K, Fujiwara Y, Nezu K.** 1988. *In vitro* antitumor activity of mitomycin C derivative (RM49) and a new anticancer antibiotic (FK973) against lung cancer cell lines determined by tetrazolium dye (MTT) assay. *Cancer Chemother Pharmacol*. **22:** 246-250.

**Horner WE, Helbling A, Salvaggio JE, Lehrer SB.** 1995. Fungal Allergens. *Clin Microbiol Rev*. **2:** 161–179.

**Hortelano IB, Pérez S, Galiano S.** 2017. Trypanothione Reductase and Superoxide Dismutase as Current Drug Targets for *Trypanosoma cruzi*: An Overview of Compounds with Activity against Chagas Disease. *Curr Med Chem*.**24:** 1066-1138.

**Hu J, Shi X, Chen J, Mao X, Zhu L, Yu L, Shi J**. 2014. Alkaloids from *Toddalia asiatica* and their cytotoxic, antimicrobial and antifungal activities. *Food Chem*. **148**: 437-444.

**Hussain S, Hore DK**. 2007. Collection and conservation of major medicinal plants of Darjeeling and Sikkim Himalayas. *Indian J Tradit Knowl*. **6:** 352-357.

**Ignat I, Volf I, Popa VI.** 2011. A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables. *Food Chem*. **126**: 1821–1835.

**Ighodaro OM, Akinloye OA**. 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria J Med*. **54**: 287-93.

**Jaffe CL, Grimaldi G, McMahon-Pratt D.** 1984. The cultivation of cloning of *Leishmania*. *In*: Morel, C.M. (Ed.), genes and antigens of parasites: A Laboratory Manual, Fundaao Oswaldo Cruz, Rio de Janeiro, Brazil. 47-91.

**Jafroodi Sk, Farazmand A, Amin M, Doroodgar A, Shirzadi M, Razavi M. 2015.** Methanolic extract's activity of *Artemisia absinthium, Vitex agnus-castus* and *Phytol acaamerica against Leishmania major*; *in vitro* and *in vivo*. *Int Arch Health Sci*. **2:** 69-74

**Jaganathan SK, Vellayappan MV, Narasimhan G, Supriyanto E.** 2014. Role of pomegranate and citrus fruit juices in colon cancer prevention. *World J Gastroenterol*.**20**:4618–4625.

**Jahn SAA**. 1996. On the introduction of a tropical multipurpose tree to China traditional and potential utilisation of *Moringa oleifera* Lamark. *Senckenb Biol*. **75**: 243-254.

**Jain SK.** 1994. Ethnobotany and research in medicinal plants in India. *Ethnobot. Search New Drugs*.**185**: 153-68.

**Jain PK, Pandey A**. 2016. The wonder of Ayurvedic medicine-Nyctanthes arbortristis. *Int J Herb Med*. **4**: 9-17.

**Jain DL, Baheti AM, Jain SR, Khandelwal KR**. 2010. Use of medicinal plants among tribes in Satpuda region of Dhule and Jalgaon districts of Maharashtra- An ethnobotanical survey. *Indian J Tradit Knowl*. **9**: 152-157.

**Jeeva S, Mishra BP, Venugopal N, Kharlukhi L, Laloo RC.** 2006. Traditional knowledge and biodiversity conservation in the sacred groves of Meghalaya. *Indian J TraditKnowl*. **5:**563-568.

**Jeyaprakash K, Ayyanar M, Geetha KN, Sekar T.** 2011. Traditional uses of medicinal plants among the tribal people in Theni districts (Western Ghats), Southern India. *Asian Pac J Trop Biomed*. **1:** 20-25.

**Joshi AB, Banjara MR, Pokhrel S, Jimba M, Singhasivanon P, Ashford RW**. 2006. Elimination of visceral leishmaniasis in Nepal: pipe-dreams and possibilities. *Kathmandu Univ Med J*. **4**: 488–496.

**Jothy SL, Zakariah Z, Chen Y, Sasidharan S.** 2012. In vitro, in situ and *in vivo* studies on the anticandidal activity of *Cassia fistula* seed extract. *Molecules*. **17:**6997–7009.

**Just MJ, Recio MC, Giner RM, Cueller MU, Manez S, Billia AR, Rios JL**. 1998. Antiinflammatory activity of unusual lupine saponins from *Bupleurum fruticescens*. *Planta med*. **64**: 404-407.

**Kala CP**. 2005. Current status of medicinal plants used by traditional vaidyas in Uttaranchal State of India. *Ethnobot Res Appl*. **3:** 267-278.

**Kala CP, Dhyani PP, Sajwan BS**. 2006. Developing the medicinal plants sector in northern India: challenges and opportunities. *J Ethnobiol Ethnomed*. **2:** 32.

**Kalpana S, Moorthi S, Kumara S.** 2013. Antimicrobial activity of different extracts of leaf of *Moringa oleifera* (Lam) against gram positive and gramnegative bacteria. *Int J Curr Microbiol App Sci*. **2:** 514-518.

**Kar K, Mukerji K, Naskar K, Bhattacharya A, Ghosh DK**. 1990. *Leishmania donovani*: a chemically defined medium suitable for cultivation and cloning of promastigotes and transformation of amastigotes to promastigotes. *J Protozool*. **37:** 277-279.

**Katewa SS, Chaudhary BL, Jain A, Galav PK**. 2003. Traditional uses of plant biodiversity from Aravalli hills of Rajasthan. *Indian J TraditKnowl*. **2**:1-7.

**Kaur R, Kaur J, Kaur S, Joshi J.2016**. Evaluation of the antileishmanial efficacy of medicinalplant *Chenopodium album* Linn. against experimental visceral leishmaniasis. *Int J Pharm Pharm Sci*. **8**: 227-231.

**Kazemi E, Talari S, Hooshyar H.** 2007. The effects of an alcoholic extract of *Berberis vulgaris* on cutaneous leishmaniasis (*L.major*) in Balb/c Mice.*J SchoolPublic Health Inst Pub Health Res*. **5:** 35-42.

**Khan MS, Ahmad I**. 2019. Herbal medicine: current trends and future prospects. *In* New look to phytomedicine. pp. 3-13. Academic Press.

**Khan MA, Ashfaq MK, Zuberi HS, Mahmood MS, Gilani AH**. 2003. The *in vivo* antifungal activity of the aqueous extract from *Nigella sativa* seeds. *Phytother Res*. **7**: 183-6.

**Khatun MR, Rahman AHMM.** 2019. Ethnomedicinal uses of plants by Santal tribal peoples at Nawabganj upazila of Dinajpur district, Bangladesh. *Bangladesh J Plant Taxon*. **26:** 117–126.

**Kheirabadi KP, Dehkordi SS, Kheibari P. 2015.** Effect of *Kelussia odoratissima* Mozaff. essential oil on promastigote form of *Leishmania major (in vitro). J Herb Med Pharmacol*. **4:** 10-14.

**Khongsai M, Saikia SP, Kayang H.** 2011. Ethnomedicinal plants used by different tribes of Arunachal Pradesh. *Ind J Trad Knowl*.**10**: 541-546.

**Khoshaban F, Ghaffarifar F, Mahammoudzadeh Poornaki A, Ghazanfari T, Naseri M, Khamesipoor A, Naeini A, Mojabi FS. 2011.** Evaluation treatment of C57BL/6 and out bred SW mice. *Pathobiol Res*. **14**: 25-34.

**Kichua M, Malewskaa T, Aktera K, Imchenb I, Harringtona D, Kohena J, Vemulpada SR, Jamie JF.** 2015. An ethnobotanical study of medicinal plants of Chungtia village, Nagaland, India. *J Ethnopharmacol*. **166:** 5-17.

**Killick-Kendrick R**. 2002. Phlebotomine sand flies: biology and control. *In*: Farrell JP, ed. *Leishmania*. Dordrecht: Kluwer Academic. 33–43.

**Kim KT, Yoo KM, Lee JW, Eom SH, Hwang IK, Lee CY.** 2007. Protective effect of steamed american ginseng (*Panax quinquefolius*L.) on V79-4 cells induced by oxidative stress. *J Ethnopharm*. **111**: 443-445.

**Kima PE**. 2007. The amastigote forms of *Leishmania* are experts at exploiting host cell processes to establish infection and persist. *Int J Parasitol*. **37**:1087–1096.

**Klejdus B, Vacek J, Benesová L, Kopecký J, Lapcík O, Kubán V**. 2007. Rapid-resolution HPLC with spectrometric detection for the determination and identification of isoflavones in soy preparations and plant extracts. *Anal Bioanal Chem*. **389**: 2277–2285.

**Kniemeyer O, Schmidt AD, Vödisch M, Wartenberg D, Brakhage AA.** 2011. Identification of virulence determinants of the human pathogenic fungi *Aspergillus fumigatus* and *Candida albicans* by proteomics. *Int J Med Microbiol*. **301**: 368-377.

**Köhler JR, Hube B, Puccia R, Casadevall A, Perfect JR.** 2017. Fungi that infect humans. *Microbiol Spectr*. **5:** 1-7

**Kongkathip B**, **Sutthiprabha S, Yoosook C, Mongkolsook Y, Kongkathip N.** 2010. Determination of a pyranocoumarin and three carbazole compounds in *Clausena excavata* by RP-HPLC. *J Chromatographic Sci*. **48:** 445-449.

**Kooli I, Marrakchi W, Toumi A, Aouam A, Baba H, Chakroun M.** 2021. Unusual occurrence of hemophagocytic lymphohistiocytosis in HIV-positive person with visceral leishmaniasis. *HIV AIDS Rev*. **4:** 311-313.

**Krings U, Berger RG**. 2001. Antioxidant activity of roasted foods. *Food Chem*. **72**: 223-229.

**Krinsky NI.** 2002. Mechanism of action of biological antioxidants. *Procceding for the Society of Experimental Biology and Medicine*. **200**: 248–54.

**Kumar M, Sarma DK, Shubham S, Kumawat M, Verma V, Singh B**, **Nagpal R, Tiwari RR.** 2021. Mucormycosis in COVID-19 Pandemic: Risk Factors and Linkages. *Curr Res Microb Sci*. **2**: 100057.

**Kumar R, Saha A, Saha D**. 2012. A new antifungal coumarin from *Clausena excavata*. *Fitoterapia*. **83**: 230–233.

**Kumar V, Kesari S, Kumar AJ, Dinesh DS, Ranjan A, Prasad M, Sinha NK, Kumar R, Das P.** 2009. Vector density and the control of kala-azar in Bihar, India. *Mem Inst Oswaldo Cruz*.**104**: 1019–1022.

**Kumari R, Rathi B, Rani A, Bhatnagar S.** 2013. *Rauwolfia serpentina* L. Benth. Ex Kurz.: Phytochemical, pharmacological and therapeutic aspects. *Int J Pharm Sci Rev Res*. **23:** 348-355.

**Laemmli UK**. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. **227**: 680–685.

**Lapornik B, Prošek M, GolcWondra A.** 2005. Comparison of extracts prepared from plant by-products using different solvents and extraction time. *J Food Eng*. **71**: 214–222.

**Lastra-Gonzalez G, Manrique CM, Govindarajan G, Whaley-Connell A, Sowers JR.** 2005. Insights into the emerging cardiometabolic prevention and management of diabetes mellitus. *Expert Opin Pharmacother*. **6:** 2209–2221.

**Lewis RE.** 2011. Current concepts in antifungal pharmacology. *Mayo Clinic Proc*. **86**: 805–817.

**Li ZJ, Guo X, Dawuti G, Aibai S**. 2015. Antifungal activity of ellagic acid *in vitro* and *in vivo*. *Phytother Res*. **29**: 1019-25.

**Lindoso JAL, Barbosa RN, Posada-Vergara MP, Duarte MIS, Oyafuso LK, Amato VS, Goto H.**2009.Unusual manifestations of tegumentary leishmaniasis in AIDS patients from the New World. *Br J Dermatol*. **160:** 311-318.

**Lohse C.** 2002. Synthesis and conformational analysis of all eight diastereoisomers of 5-Methylyohimbane. *Helv Chim Acta*. **85:** 945–961.

**Lourenço SC, Moldão-Martins M, Alves VD**. 2019. Antioxidants of natural plant origins: From sources to food industry applications. *Molecules*. 22: 4132.

**Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J.Randall.1951.** Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. **193**: 265-275.

**Lu M, Li T, Wan J, Li X, Yuan L, Sun S.** 2017. Antifungal effects of phytocompounds on *Candida* species alone and in combination with fluconazole. *Int J Antimicrob Agents*. **49:** 125–136.

**Lucas DS, Sekhar RAR.** 2000. A review of experimental studies on antihepato-protective activity of certain medicinal plants used in Ayurveda. *Phytomedicine*. Supplement-II: **2000:**23.

**Macêdo CG, Castro KCF, da Silva EO.** 2021. Antileishmanial activity of the genus *Piper*: A systematic review. *Eur J Med Chem*. **32:** 21-44.

**Mahida Y, Mohan JSS.** 2007. Screening of plants for their potential antibacterial activity against *Staphylococcus* and *Salmonella* sp. *Natural Product Radiance*. **6:** 301-305.

**Mahmoudvand H, Ezzatkhah F, Sharifiar F, Sharifi I, Dezaki ES. 2015.** Antileishmanial and cytotoxic effects of essential oil and methanolic extract of *Myrtusco mmunis* L. *Korean J. Parasitol*. **53:** 21-27.

**Mahmoudvand H, Sharififar F, Sharifi I, Ezatpour B, FasihiHarandi M, Makki MS.** 2014. *In vitro* inhibitory effect of *Berberis vulgaris* (Berberidaceae) and its main component, berberine against different *Leishmania* species.*Iran J Parasitol*. **9**: 28-36.

**Majumdar D, Deka SN, Pujari D, Das PK.** 2013. Traditional knowledge adopted by the farmers for management of rice pests in North bank plain zone of Assam. *Indian J Tradit Knowl*. **12:** 725- 729.

**Makkar HPS, Becker K.** 1997. Nutrients and anti-quality factors in different morphological parts of the *Moringa oleifera* tree. *J Agric Sci Cambridge*.**128:** 311-322.

**Mann J**. 1978. Secondary Metabolism. Oxford University press, London. 154.

**Mann S, Frasca K, Scherrer S, Henao-Martínez AF, Newman S, Ramanan P, Suarez JA.** 2021. A review of leishmaniasis: current knowledge and future directions. *Curr Trop Med Rep*. **8**: 121-32.

**Manosroi A, Saraphanchotiwitthaya A, Manosroi J**. 2005. *In vivo* immunomodulating activity of wood extracts from *Clausena excavata* Burm.f. *J Ethnopharmcol*.**102**: 5-9.

**Mao AA, Hynniewta TM.** 2011. Plants used as agricultural season's indicator by MaoNaga tribe, Manipur, India. *Indian J Tradit Knowl*. **10:** 578-580.

**Mao AA, Roy DK.** 2016. Ethnobotanical studies in Northeast India: A review. In: Jain AK ed. Indian Ethnobotany: Emerging trends. Scientific publisher, India. 99-112.

**Mao AA.** 2002. *Oroxylum indicum* Vent.- A potential anticancer medicinal plant. *Indian J Tradit Knowl*. **1:** 17-21.

**Marjorie C**. 1996. Plant products as antimicrobial agents. *Clincal Microbiol Rev*. **12:** 564-582.

**Marklund S**, **Marklund G.** 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*. **47:** 469-474.

**Martínez DY, Verdonck K, Kaye PM, Adaui V, Polman K, Llanos-Cuentas A, Dujardin JC, Boelaert M.** 2018.Tegumentary leishmaniasis and coinfections other than HIV. *PLoSNegl Trop Dis*. **12**:1-20.

**Martin-Montes A, Santivañez-Veliz M, Moreno-Viguri E, Martín-Escolano R, Jiménez-Montes C, Lopez-Gonzalez C, Marín C, Sanmartín C, Sánchez RG, Sánchez-Moreno M, Pérez-Silanes S.** 2017. *In vitro* antileishmanial activity

and iron superoxide dismutase inhibition of arylamine Mannich base derivatives. *Parasitology*. **144**: 1783-1790.

**Martins N, Barros L, Henriques M, Silva S, and Ferreira ICFR.** 2015. *In vivo* anti-*Candida* activity of phenolic extracts and compounds: Future perspectives focusing on effective clinical interventions. *Biomed Res Int*. **2015:** 1-14.

**Martins N, Ferreira ICFR, Barros L, Silva S, Henriques M.** 2014. Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment. *Mycopathologia*. **177:**223–240.

**Marty P, Rosenthal E.** 2002. Treatment of visceral leishmaniasis: a review of current treatment practices. *Expert Opin Pharmacother*.**3:** 1101-1108.

**Marwaha N, Sarode R, Gupta RK, Gerewal G, Dash S.** 1991. Clinico-hematological characteristics in patients with Kala-azar. A study from north-west India. *Trop Geogr Med*. **43**: 357-362.

**Masoko P, Picard J, Howard RL, Mampuru LJ, Eloff JN.** 2010. *In vivo* antifungal effect of *Combretum* and *Terminalia* species extracts on cutaneous wound healing in immunosuppressed rats. *Pharm Biol*. **48**: 621–632.

**Maspi N, Ghafarifar F, Bahrami A, Bastaminejad S, Shamsi M. 2010.** Evaluation of leishmanicidal effect of watery and ethanolic flowers *Calendula officinalis* extract on promastigotes of *Leishmani major* (MRHO/IR/75/ER) *in vitro*. *J Ilam Univ Med Sci*. **18:** 28-23.

**Maynard CL, Weaver CT**. 2008. Diversity in the contribution of interleukin-10 to T-cell-mediated immune regulation. *Immunol Rev*.**226**: 219-233.

**Mayne PD.** 1994. Clinical chemistry in diagnosis and treatment. ELBS, UK.

**McNeely TB, Rosen G, Londner MV, Turco SJ.** 1989. Inhibitory effects on protein kinase C activity by lipophosphoglycan fragments and glycosylphosphatidylinositol antigens of the protozoan parasite *Leishmania*. *Biochem J*. **259:** 601-604.

**Mesa LE, Lutgen DVP, Vélez IV, Restrepo AM, Isabel Ortiz I, Robledo SM.** 2017. *In vitro* and *in vivo* antileishmanial activity of *Artemisia annua* L. leaf powder and its potential usefulness in the treatment of uncomplicated cutaneous leishmaniasis in humans. *Rev Soc Bras Med*. **50:** 52-60.

**Mesfin K, Tekle G, Tesfay T.** 2013. Ethnobotanical study of traditional medicinal plants used by indigenous people of Gemad District, Northern Ethiopia. *J Med Plants Stud.***1**: 32-37.

**Miceli MH, Díaz JA, Lee SA**. 2011. Emerging opportunistic yeast infections. *Lancet Infect Dis*. **11:** 142-151.

**Mishra BN, Mishra MK.** 1983. In introductory practical biostatistics. Naya Prakash. Calcutta.

**Mishra BB, Tiwari VK.** 2011. Natural products: An evolving role in future drug discovery. *Eur J Med Chem*. **46**: 4769-4807.

**Monzote L, García M, Montalvo AM, Scull R, Miranda M.** 2010. Chemistry, cytotoxicity and antileishmanial activity of the essential oil from *Piper auritum*. *Mem Inst Oswaldo Cruz*.**105:** 168–173.

**Moore RD, Chaisson RE.** 1996. Natural history of opportunistic disease in an HIV infected urban clinical cohort. *Ann Intern Med*. **124:** 633-642.

**Moreno MIN, Isla MI, Sampietro AR, Vattuone MA**. 2000. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. *J Ethnopharmacol*. **71**: 109-114.

**Morrow CD, Flory B, Krassner SM.** 1980. Polyamines in the hemoflagellate, *Leishmania donovani*: evidence for spermine in the amastigote stage. *Comp Biochem Physiol*. **66:** 307-311.

**Mosmann T**. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. **65**: 55–63.

**Murray HW.** 2001. Clinical and experimental advances in treatment of visceral leishmaniasis. *Antimicrob Agents Chemother*. **45:** 2185-2197.

**Murthy KM, Narayanappa M.** 2015. *In vitro* study of antibacterial activity of leaf and root extract of *Rauvolfia serpentina* against gram positive and negative bacteria strains. *Int J Rec Res Interdiscipl Sci*. **2:**33-37.

**Nadkarni AK.** 1954. Indian Materia Medica, Voll, 3rd ed. Popular Prakashan Pvt. Ltd. Bombay & Dhootapapeshwar Prakashan Ltd. Panvel.

**Nair VD, Panneerselvam R, Gopi R.** 2012. Studies on methanolic extract of *Rauvolfia* species from Southern Western Ghats of India—*in vitro* antioxidant properties, characterisation of nutrients and phytochemicals. *Ind Crop Prod.***39:** 17–25.

**Nandi RP**. 2005. Jalpaiguri ZilarBanousadhi*In:* Jalpaiguri Zila SanskaranKiratbhumi, Jalpaiguri. **2:** 263-275.

**Nasser AMAG, Court WE.** 1984. Stem bark alkaloids of *Rauwolfia caffra*. *J Ethnopharmacol*.**11:** 99–117.

**Nath M, Choudhury MD.** 2009. Ethnoveterinary practice by Hmar tribe in Cacher District, Assam. *Ethnobot*.**21:** 61-65.

**Navie S, Csurhes S**. 2010. Weed risk assessment: *Moringa oleifera*. Biosecurity Queensland department of employment, economic development and innovation. Brisbane.

**Negi JS, Bisht VK, Bhandari AK, Bisht DS, Singh P, Singh N.** 2014. Quantification of reserpine content and antibacterial activity of *Rauvolfia serpentina* (L.) Benth. ex Kurz. *Afr J Microbiol Res*. **8:** 162-166.

**Neto J, Pereira M, Oliveira J, Rocha-Bezerra L, Lopes T, Costa H, Sousa D, Rocha B, Grangeiro T, Freire J, Monteiro Moreira A, Lobo M, Brilhante R, Vasconcelos I.** 2017. A Chitin-binding protein purified from *Moringa oleifera* seeds presents anticandidal activity by increasing cell membrane permeability and reactive oxygen species production. *Front Microbiol*. **8:** 980.

**Ningombam DS, Ningthoujam SS, Singh PK, Singh OB.** 2012. Legacy of kum dye: A case study with the Meitei community in Manipur, Northeast India. *Ethnobot Res Appl*. **10:** 561-570.

**Nishikimi M, Rao NA, Yagi K.** 1972.The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun*. **46**: 849–853.

**Nobori T, Miurak K, Wu DJ, Takabayashik LA, Carson DA**. 1994. Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature*. **46**: 753-756.

**Nyarko AA, Addy ME.** 1990. Effects of aqueous extract of *Adenia cissampeloides* on blood pressure and serum analyte of hypertensive patients. *Phytotherapy Res.***4:**25-28.

**Okabe N, Adachi Y.** 1998. 1H-Indole-3-propionic Acid. *Acta Crystallogr C Struct Chem*. **54:** 386–387.

**Okwu DE, Okwu ME**. 2004. Chemical composition of *Spondias mombin* Linn. plant parts. *J Sustain Agric Environ*. **6:** 140-147.

**Okwu DE**. 2001. Evaluation of chemical composition of medicinal plants belonging to Euphorbiaceae. *Pak Vet J*. **14:** 160-162.

**Okwu DE.** 2004. Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. *J Sustain Agric Environ*. **6**: 30-37.

**Olliaro P, Bryceson AD.** 1993. Practical progress and new drugs for changing patterns of Leishmaniasis. *Parasitol Today*. **9**: 323-328.

**Omino EA, Kokwaro JO**. 1993. Ethnobotany of Apocynaceae species in Kenya. *J Ethnopharmacol*. **40:** 167–180.

**Oskuee R, Jafari M, Farzad SA, Ramezani M.** 2012. *In vitro*Leishmanicidal activity of *Calotropis gigantea* and its fractions against *Leishmania major*. *J Med Plant Res*. **6**: 3977-3983.

**Ostan I, Saglam H, Limoncu ME, Ertabaklar H, OzensoyToz S, Ozbel Y, Ozbilgin A.** 2007. *In vitro* and *in vivo* activities of *Haplophyllum myrtifolium* against *Leishmania tropica. New Microbiol*. **30:** 439-444.

**Ozbilgin A, Durmuskahya C, Kayalar H, Ertabaklar H, Gunduz C, Ural IO, Zeyrek F, Kurt O, Cavus I, Balcıoglu C, Toz SO, Ozbel Y.** 2014. Antileishmanial activity of selected Turkish medicinal plants. *Trop J Pharm Res.***13:** 2047-2055.

**Pappas PG, Alexander BD, Andes DR.** 2010. Invasive fungal infections among organ transplant recipients: results of the transplant-associated infection surveillance network (TRANSNET). *Clin Infect Dis*. **50**: 1101–1111.

**Patel RM, Patel NJ.** 2011. *In vitro* antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. *J Adv Pharm Educ Res*. **1**: 52-68.

**Patterson TF, Thompson GR, Denning DW, Fishman JA, Hadley S, Herbrecht R, Kontoyiannis DP, Marr KA, Morrison VA, Nguyen MH, Segal BH, Steinbach WJ, Stevens DA, Walsh TJ, wingard JR, Young JA, Bennett JE.** 2016. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infectious Diseases Society of America. *Clin Infect Dis.* **63:** 1–60.

**Paul S, Devi N, and Sarma GC.** 2013. Ethnobotanical utilization of some medicinal plants by Bodo people of Manas Biosphere reserve in the treatment of malaria. *Int Res J Pharm*. **4:** 102-105.

**Payyappallimana U, Fadeeva Z**. 2013. Traditional Knowledge and Biodiversity. United Nations University-IAS. Yokohama.

**Perfect JR, Casadevall A.** 2002. Cryptococcosis. *Infect DisClin North Am*. **16**: 837-874.

**Pfaller MA, Diekema DJ**. 2002. Role of sentinel surveillance of candidemia: Trends in species distribution and antifungal susceptibility. *J Clin Microbiol*. **40**: 3551-3557.

**Pfaller MA, Diekema DJ**. 2007. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev.* **20**: 133-163.

**Phillipson JD, Wright CW.** 1996. Plants with antiprotozoal activity. *In* Trease and Evans Pharmacognosy, 14th edn., WB Saunders Company. London.

**Palatnik-de-Sousa, C. B.** 2008. Vaccines for leishmaniasis in the fore coming 25 years. *Vaccine*. **26**. 1709-1724.

**Poddar A, Banerjee A, Ghanta S, Chattopadhyay S.** 2008. *In-vivo* efficacy of calceolarioside A againstexperimental visceral leishmaniasis. *Planta Medica*. **74:** 503-508.

**Pourmorad F, Hosseinimehr SJ, Shahabimajd N**. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr J Biotechnol*. **5**: 1142-1145.

**Prain D.** 1903. Bengal Plants. Botanical Survey of India, Kolkata. Vol. I-II.

**Prakash V, Aggarwal A.** 2010. Traditional uses of ethnomedicinal plants of lower foot hills of Himachal Pradesh-I. *Ind J Tradit Knowl*. **9:** 519-521.

**Procop GW (ed.).** 2017. Koneman'scoloratlas and textbook of diagnostic Microbiology, Philadelphia: Wolters. Kluwer Health.

**Puongtip K, Ampai P, Narong N, Prapadsorn P, Vichai R.** 2011. Acute and repeated dose 90-day oral toxicity studies of *Clausena excavata* extract in rats. *J Pharm Biomed Sci*. **12**: 1-7.

**Queiroz ACD, Dias TLMF, Matta CBBD, Silva LHAC, Araújo JX, Araújo GB, Moura FBP, Alexandre-Moreira MS.** 2014. Antileishmanial Activity of medicinal plants used in endemic areas in Northeastern Brazil. *Evid Based Complement Alternat Med.* **2014:** 1-9.

**Rahman HS, Rasedee A, Abdul AB, Zeenathul NA, Othman HH,Yeap SK, How CW, Hafiza N. 2014.** Zerumbone-loaded nanostructured lipid carrier induces G2/M cell cycle arrest and apoptosis via mitochondrial pathway in a human lymphoblastic leukemia cell line. *Int J Nanomed.* **9**: 527–538.

**Rajagopala S, Dutta U, Chandra KP, Bhatia P, Varma N, Kochhar R**. 2008. Visceral leishmaniasis associated hemophagocytic lymphohistiocytosis–case report and systematic review. *J Infection*. **56**: 381-8.

**Ram VJ, Haque N, Guru PY**. 1992. Chemotherapeutic agents XXV: synthesis and leishmanicidal activity of carbazol pyrimidines. *Eur J Med Chem*. **27**: 851-855.

**Ranjini HS, Udupa EG, Thomas JM.** 2015. Article angiotensin converting enzyme (ACE): Inhibition of sheep kidney and lung ACE *in vitro* by *Rauwolfia serpentina* and *Allium sativum.Sch J Appl Med Sci*. **3:** 1936-1940.

**Rao RR, Jamir NS.** 1982. Ethnobotanical studies in Nagaland-I: Medicinal plants. *Econ Bot*. **36:** 176-181.

**Rao RR**. 1981. Ethnobotany of Meghalaya: Medicinal plants used by Khasi and Garo tribes. *Econ Bot*. **35**: 1-9.

**Rao V, Mans D, Rao L**. (Eds.) 2020. Phytochemicals in Human Health. *Intech Open*. 77908.

**Rathi G, Siddiqui SI, & Pham Q.** 2020. Nigella sativa seeds based antibacterial composites: a sustainable technology for water cleansing-a review. *Sustainable Chemistry and Pharmacy*. **18**. 100332.

**Rathore B, Paul B, Chaudhary BP, Saxena AK, Sahu AP, Gupta YK.** 2007. Comparative studies of different organs of *Nyctanthes arbortristis* in modulation of cytokines in murine model of arthritis. *Biomed Environmental Sci*. **20**: 154-159.

**Ray JC**. 1932. Cultivation of various *Leishmania* parasites on solid medium. *Ind J Med Res.***20**: 355-357.

**Raychaudhury B, Banerjee S, Gupta S, Singh RV, Datta SC**. 2005. Antiparasitic activity of a triphenyl tin complex against *Leishmania donovani*. *Acta Tropica.* **95:** 1–8.

**Rees PH, Keating MI, Kager PA, Hockmeyer WT**. 1980. Renal clearance of pentavalent antimony (sodium stibogluconate). *Lancet*. **2**: 226–229.

**Ribeiro TG, Chávez-Fumagalli MA, Valadares DG, Franca JR, Lage PS, Duarte MC, Andrade PHR, Martins VT, Costa LE, Arruda ALA, Faraco AAG, Coelho EAF, Castilho RO.** 2014. Antileishmanial activity and cytotoxicity of Brazilian plants. *Exp Parasitol*. **143:** 60-68.

**Ridley HN.** 1922. The Flora of the Malay Peninsula: Apetalae. L. Reeve and co., ltd.

**Rio DD, Mateos AR, Spencer JPE, Tognolini M, Borges G, Crozier A.** 2013. Dietary (Poly) phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. *Antioxid Redox Signal*. **18:** 1818—1892.

**Rittig MG, Bogdan C**. 2000. *Leishmania*-host-cell interaction: complexities and alternative views. *Parasitol Today*. **16**: 292-297.

**Rocha LG, Almeida JR, Macêdo RO, BarbosaFilho JM**. 2005. A review of natural products with anti-*Leishmanial* activity. *Phytomedicine*. **12**: 514–535.

**Rodrigues ML, Nosanchuk JD.** 2020. Fungal diseases as neglected pathogens: A wake-up call to public health officials. *PLoSNegl Trop Dis*. **14:** 1-9.

**Rogers TR.** 2006. Antifungal drug resistance: limited data, dramatic impact? *Int J Antimicrob Agents*. **27**: 7-11.

**Rosen WE, Shoolery JN.** 1961. *Rauwolfia* Alkaloids. XLI. Methyl Neoreserpate, an isomer of Methyl reserpate. Part 3. Conformations and NMR. Spectra. *J Am Chem Soc*. **83:** 4816-4819.

**Ross R.** 1903. Note on the bodies recently described by Leishman and Donovan. *Br Med J*. **2**: 1261–1262.

**Roy S.** 2015. An ethnobotanical study on the medicinal plants used by Rajbanshis of Coochbehar district, West Bengal, India. *J Med Plants Stud*. **3:** 46-49.

**Roy SK**. 2005. Tribes, Education and Gender Question, Northern Book Center, New Delhi.

**Sacks D, Kamhawi S.** 2001. Molecular aspects of parasite-vector and vector-host interaction in Leishmaniasis. *Annu Rev Microbiol*. **55**: 453-483.

**Sadeghi-Nejad B, Saki J, Azish M**. 2014. Effect of aqueous *Allium cepa* and *Ixora brachiata* root extract on *Leishmania major* promastigotes, *JundishapurJ Nat Pharm Prod*.**9**: e15442.

**Sadeghi-Nejad B, Saki J, Khademvatan S, Nanaei S.** 2011. *In vitro* antileishmanial activity of the medicinal plant *Saturejakhuzestanica* Jamzad. *J Med Plant Res*.**5**: 5912-5915.

**Saha D, Dasgupta S, Saha A.** 2005b. Control of foliartea diseases by leaf extracts of *Polyalthialongifolia. J Mycol Plant Pathol*. **35**: 132-136.

**Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S, Mansor SM.** 2011. *Invitro* and *invivo* anticandidal activity of *Swietenia mahogani* methanolic seed extract. *Trop Biomed*. **28:** 132–137.

**Saito K, Murray HW.** 2020. Chiclero's ulcer. *Int J Infect Dis*. **94:** 12–13.

**Sakong P, Khampitak T, Chaon U, Pinitsoontorn C, Sriboonlue P, Yongvanit P, Boonsiri P.** 2011. Antioxidant activity and bioactive phytochemical contents of traditional medicinal plants in northeast Thailand. *J Med Plants Res*. **5:** 6822-6831.

**Saleem M, Kim HJ, Ali MS, Lee YS.** 2005. An update on bioactive plant lignans. *Natural Product Reports*. ***22***: 696–716.

**Salehabadi A, Karamian M, Farzad MH, Namaei MH.** 2014. Effect of root bark extract of *Berberis vulgaris* L. on *Leishmania major* on Balb/c mice. *Parasitol Res*. **113:** 953-957.

**Salvaggio J, Aukrust L.** 1981. Mold-induced asthma. *J Allergy Clin Immunol*. **68:** 327–346.

**Samaranayake YH, Samaranayake LP**. 1994. *Candida krusei*: Biology, epidemiology, pathogenicity and clinical manifestations of an emerging pathogen. *J Med Microbiol*. **41:** 295-310.

**Samson RA**. 2007. Diagnostic tools to identify black aspergilli. *Stud Mycol*. **59:** 129-145.

**Sanglard D**. 2003. Resistance and tolerance mechanisms to antifungal drugs in fungal pathogens. *Mycologist*. **17**:74-78.

**Santos VR, Pereira EMR.** 2018. Antifungal activity of Brazilian medicinal plants against *Candida*species. *In* Sandei D. (ed) *Candida albicans*. *Intech Open*. 19-47.

**Sasmal D, Das S and Basu SP**. 2007. Phytoconstituents and therapeutic potential of *Nyctanthes arbor-tristis* Linn. *Pharmacog Rev*. **1**: 344-349.

**Schmiedel Y, Zimmerli S**. 2016. Common invasive fungal diseases: an overview of invasive candidiasis, aspergillosis, cryptococcosis, and pneumocystis pneumonia. *Swiss Med Wkly*. **146:** 1-12

**Schwartz IS, Govender NP, Corcoran C, Dlamini S, Prozesky H, Burton R, Mendelson M, Taljaard J, Lehloenya R, Calligaro G, Colebunders R, Kenyon C.** 2015. Clinical characteristics, diagnosis, management, and outcomes of disseminated emmonsiosis: A retrospective case series. *Clin Infect Dis*. **61:** 1004-1012.

**Scorzoni, L, Sangalli-Leite, F, Singulani, JL, de Paula e Silva, ACA, Costa-Orlandi, CB, Fusco-Almeida, AM, Mendes-Giannini, MJS.** 2016. Searching new antifungals: The use of *in vitro* and *in vivo* methods for evaluation of natural compounds. *J Microbiol Methods*. **123**: 68-78.

**Seepe HA, Lodama KE, Sutherland R, Nxumalo W, Amoo SO**. 2020. *In vivo*antifungal activity of South African medicinal plant extracts against *Fusarium*pathogens and their phytotoxicity evaluation. *Plants*. **9:** 1668-1685.

**Serafini M**. 2006. The role of antioxidants in disease prevention. *Medicine*. **34**: 533-535.

**Shamma M, Richey JM.** 1963. The stereochemistry of the heteroyohimbine alkaloids. *J Am Chem Soc*. **85:** 2507–2512.

**Shan B, Cai YZ, Sun M, Corke H.** 2005. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *J Agric Food Chem*. **53:** 7749-7759.

**Shankar R, Lavekar GS, Deb S, Sharma BK**. 2012.Traditional healing practice and folk medicines used by Mishing community of North East India. *J Ayurveda Integr Med*. **3**: 124-129.

**Sharif NWM, Mustahil NA, Noor HSM, Sukari MA, Rahmani M, Yap YHT, Ee GCL**. 2011. Cytotoxic constituents of *Clausena excavata*. *Afr J Biotechnol*. **10:** 16337-16341.

**Sharma S, Ahmed A, Singh M, Gupta S.** 2013. Anti-fungal evaluation of plant *Nyctanthes arbour-tristis*Linn. *Int J Adv Drug Deliv*. **3**:1-7.

**Shier WT**. 1983. An undergraduate experiment to demonstrate the use of cytotoxic drugs in cancer chemotherapy. *Am J Pharm Educ*. **47:** 216-220.

**Shrivastava R, Bharadwaj A.** 2018. *Nyctanthesarbor-tristis* an important medicinal plant of Madhya Pradesh State - A Review. *UK J Pharm Biosci*. **6:** 10-15.

**Siddique I, Anis M, Jahan AA.** 2006. Rapid multiplication of *Nyctanthesarbor-tristis* L. through *in vitro* axillary shoot proliferation. *World J Agric Sci*. **2:** 188-192.

**Simon-Nobbe B, Denk U, Poll V, Rid R, Breitenbach M.** 2008. The spectrum of fungal allergy. *Int Arch Allergy Immunol*.**145:** 58–86.

**Singh B, Barthakur SK, Phukan SJ, Sinha BK.** 2012. Assessing ethnobotanical values and threat status of wild Asparagus (*Stemona tuberosa* Lour.): A case study in eastern Himalaya, India. *Int J Conserv Sci*. **3:** 319-324.

**Singh R, Singh SK, Arora S.** 2007. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. *Food Chem Toxicol*. **45:** 1216-1223.

**Singh OP, Sundar S.** 2015. Developments in diagnosis of visceral leishmaniasis in the elimination era. *J Parasitol Res*. **2015**: 239469.

**Singh U, Lahiri N**. 2010. *Ancient India: New Research*, Oxford University Press, New Delhi.

**Sinhababu A, Banerjee A.** 2013. Ethno-botanical study of medicinal plants used by tribals of Bankura district, West Bengal, India. *J Med Plants Stud*. **1:** 98-104.

**Skalski J, Limper A.** 2016. Fungal, viral, and parasitic pneumonias associated with human immunodeficiency virus. *Semin Respir Crit Care Med*. **37:** 257–266.

**Sofowara A**. 1993. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan. Nigeria. ***2*:** 365-372.

**Soliman SSM, Semreen MH, El-Keblawy AA, Abdullah A, Uppuluri P, Ibrahim AS.** 2017. Assessment of herbal drugs for promising anti-*Candida* activity. *BMC Complement Altern Med*. **17:** 257-265.

**Sosa N, Pascale JM, Jiménez AI, Norwood JA, Kreishman-Detrick M, Weina PJ, Lawrence K, McCarthy WF, Adams RC, Scott C, Ransom J, Tang D, Grogl M**. 2019. Topical paromomycin for New World cutaneous leishmaniasis. *PLoSNegl Trop Dis*. **13:**1-12.

**Stray F**. 1998. The natural guide to medicinal herbs and plants. Tiger Books International. London. 12-16

**Strohl MJ, Seikel MK.** 1965. Polyphenols of pine pollens. *Phytochem*. **4**: 383– 399.

**Sukieum S, Sang-aroon W, Yenjai C.** 2017. Coumarins and alkaloids from the roots of *Toddalia asiatica*. *Nat Prod Res*. **32:** 944-952.

**SundarS, Jha TK, Thakur CP, Mishra M, Singh VR, Buffels R**. 2002. Low-dose liposomal amphotericin B in refractory Indian visceral leishmaniasis: a multicenter study. *Am J Trop Med Hyg*. **66**: 143–146.

**Sundar S.** 2001. Drug resistance in Indian visceral leishmaniasis.*Trop Med Int Health*. **6:**849-854.

**Sunthitikawinsakul A, Kongkathip N, Kongkathip B,Phonnakhu S, Daly JW, Spande TF, Nimit Y, Napaswat C, Kasisit J, Yoosook C.** 2003. Anti-HIV-1 limonoid: first Isolation from *Clausena excavata*. *Phytother Res.***17:** 1101–1103.

**Sureshkumar P**. 2008. Anti-fungal activity of *Leptadenia reticulata* in rat animal model *in vivo*. *Int J Basic Appl Biol*. **2**: 9-13.

**Swaminath CS, Shortt HE, Anderson LAP.** 1942. Transmission of Indian kala-azar to man by the bites of *Phlebotomus argentipes*. *Ann Brun Ind J Med Res*. **30:** 473–477.

**Swarbrick JT**. 1997. Environmental weeds and exotic plants on Christmas Island. Indian Ocean: a report to Parks Australia. Parks Australia (North), Weed Science Consultancy.

**Takemura Y, Nakamura K, Hirusawa T, Ju-ichi M, Ito C, Furukawa H**.2000. Four new furanone-coumarins from *Clausena excavata*. *Chem Pharm Bull*. **48:** 582-584.

**Taleb-Contini SH, Salvador MJ, Watanabe E, Ito IY, Oliveira DC**. 2003. Antimicrobial activity of flavonoids and steroids isolated from two *Chromolaena* species. *Revista Brasileira de Ciências Farmacêuticas*. **39**: 403-408.

**Taran M, Mohebali M, Esmaeli J.**2010. *In vivo*efficacy of gum obtained *Pistacia atlantica*in experimental treatment of cutaneous leishmaniasis. *Iran J Publ Health*. **39**: 36-41.

**Taufiq-Yap YH, Peh TH, Ee GCL, Rahmani M, Sukari MA, Ali AM, Muse R**. 2007. A new cytotoxic carbazole alkaloid from *Clausena excavata*. *Nat Prod Res*. **21**: 810-813.

**Thakur CP, Narayan S, Ranjan A**. 2004. Epidemiological, clinical & pharmacological study of antimony-resistant visceral leishmaniasis in Bihar, India. *Ind J Med Res*.**120**: 166-172.

**Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H.** 2011. Phytochemical screening and extraction: A review. *Internationale Pharmacutica Sciencia.***1**: 98- 106.

**Torres-Guerrero E, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, Arenas R.** 2017. Leishmaniasis: a review. *F1000 Research*. **6**: 750.

**Tosti A, Piraccini BM, Lorenzi S.** 2000. Onychomycosis caused by non dermatophytic molds: clinical features and response to treatment of 59 cases. *J Am Acad Dermatol*. **42**: 217-224.

**Trease GE, Evans WC**. 1989. Pharmacognsy. 11th ed. Brailliar Tiridel Can. Macmillian Publishers.

**Tsaknis J, S Lalas, V Gergis, V Douroglou, V Spiliotis.** 1999. Characterization of *Moringa oleifera* variety Mbololo seed oil of Kenya. *J Agri Food Chem*. **47:** 4495-4499.

**Tsao R, Yang R.** 2003. Optimization of a new mobile phase to know the complex and real polyphenolic composition: towards a total phenolic index using high-performance liquid chromatography. *J Chromatogr A.***1018**: 29–40.

**Tuntiwachwuttiku P, Rayanil K, Taylor WC.** 2003. Chemical constituents from the flowers of *Nyctanthesarbor-tristis*. *Sci Asia*. **29:** 21-30.

**Vaidyaratnam PS.** 2010. Indian Medicinal Plants. Hyderabad: Universities Press India Pvt. Ltd. **4:** 409-410.

**Van HT, Nguyen DGM, Quynh NTA, Le VS.** 2020. Antibacterial activities of ethanolic extract of four species of Rutaceae family. *Plant Sci Today*. **7:** 463-468.

**Vandeputte P, Ferrari S, Coste AT.** 2012. Antifungal resistance and new strategies to control fungal infections. *Int JMicrobiol*. **2012:** 1-26.

**Vanloubbeeck Y, Jones DE.** 2004. The Immunology of *Leishmania* infection and the implication for vaccine development. *Ann NY Acad Sci*. **1026**: 267-272.

**Vasu K, Goud JV, Suryam A, Singara Chary MA.** 2009. Biomolecular and phytochemical analyses of three aquatic angiosperms. *Afr. J. Microbiol. Res.***3**: 418-421.

**Vickerman K, Tetley L.** 1990. Flagellar surfaces of parasitic protozoa and their role in attachment. In (ed Bloodgood RA) Ciliary and flagellar membranes. Plenum Press, New York. 267-304.

**Vieira-Araújo FM, Macedo Rondon FC, Pinto Vieira ÍG, Pereira Mendes FN, Carneiro de Freitas JC, Maia de Morais S.** 2018. Sinergism between alkaloids piperine and capsaicin with meglumineantimoniate against *Leishmania infantum*. *Exp Parasitol*. **188:** 79-82.

**Vishwanathan M, Juvekar AR.** 2010. Hepatoregenerative effects of *Nyctanthesarbor-tristis* Linn. on acetaminophen induced oxidative damage in rats. *Int J Pharma Tech Res.* **2:** 1291-1297.

**Volpedo G, Pacheco-Fernandez T, Holcomb EA, Cipriano N, Cox B, Satoskar AR.** 2021. Mechanisms of immunopathogenesis in cutaneous leishmaniasis and post kala-azar dermal leishmaniasis (PKDL). *Front Cell Infect Microbiol*. **11:** 1-16.

**Wang H, Cao G, Prior RL**. 2008. Total antioxidant capacity of fruits. *J Agri Food Chem*. **44**: 701–705.

**Wang RJ, Miller RF, Huang L.** 2017. Approach to fungal infections in HIV-infected individuals: *Pneumocystis* and beyond. *Clin Chest Med.* **38:** 465-477.

**Wang Y, Branicky R, Noë A, Hekimi S**. 2018. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *J Cell Biol*. **217**: 1915-28.

**Wangcharoen W, Gomolmanee S**. 2013. Antioxidant activity changes during hot-air drying of *Moringa oleifera* leaves. *Maejo Int J Sci Technol*. ***7***: 353-363.

**Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD.** 2017. Azole antifungal resistance in *Candida albicans* and emerging non-*albicans Candida* species. *Front Microbiol*. **7:** 1-12.

**Wheat LJ, Azar MM, Bahr NC, Spec A, Relich RF, Hage C.** 2016. Histoplasmosis. *Infect Dis Clin North Am.* **30:** 207–227.

**Wiart C, Mogana S, Khalifah S, Mahan M, Ismail S, Buckle M, Narayana AK, Sulaiman M.** 2004. Antimicrobial screening of plants used fortraditional medicine in the state of Perak, Peninsular Malaysia. *Fitoterapia.* **75:** 68-73.

**Wilson LS, Reyes CM, Stolpman M, Speckman J, Allen K, Beney J.** 2002. The direct cost and incidence of systemic fungal infections. *Value Health*. **5:** 26–34.

**Woodruff AW**. 1972. Recent work on anaemias in the tropics. *Br Med Bull*. **28:** 92–95.

**Wozencraft AO, Blackwell JM**. 1987. Increased infectivity of stationary-phase promastigotes of *Leishmania donovani*: correlation with enhanced C3 binding capacity and CR3-mediated attachment to host macrophages. *Immunology.* **60**: 559-563.

**Wu TS, Huang SC, Wu PL**. 1996. Carbazole alkaloids from stem bark of *Clausenaexcavata*. *Phytochem*. **43:** 1427-1429.

**Wu TS, Huang SC**. 1992. Clausine-D and -F, two new 4-prenylcarbazole alkaloids from *Clausenaexcavata*. *Chem Pharm Bull*. **40:** 1069-1071.

**Xin ZQ, Lu JJ, Ke CQ, Hu CX, Lin LP, Ye Y.** 2008. Constituents from *Clausenaexcavata*. *Chem Pharm Bull*. **56**:827-830.

**Yadav RNS, Agarwala M.** 2011. Phytochemical analysis of some medicinal plants. *J Phytol*. **3:** 10-14.

**Yadav SS, Bhandoria MS.** 2013. Ethnobotanical exploration in Mahendergarh district of Haryana (India). *J med Plants Res*. **7**: 1263-1271.

**Yap YHT, Peh TH, Ee GCL, Rahmani M, Sukari MA, Ali AM, Muse R.** 2007. A new cytotoxic carbazole alkaloid from *Clausena excavata.Nat Prod Res*. **21**: 810-813.

**Yasuka K.** 1978. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Arch Biochem Biophys*. **186**: 189–195.

**Yiannakopoulou EC.** 2014. Effect of green tea catechins on breast carcinogenesis: a systematic review of *in vitro* and *in vivo* experimental studies. *Eur J Cancer Prev.**23***: 84–89.

**Young IS**, **Woodside JV**. 2001. Antioxidants in health and disease. *J Clin Pathol*. **54**: 176-186.

**Yousefi R, Ghaffarifar F, Asl AD.** 2009. The effect of *Alkanna tincturia* and *Peganum harmala* extracts on *Leishmania major* (MRHO/IR/75/ER) *in vitro*. *Iran J Parasitol*. **4:** 40-47.

**Zaffer M, Ganie SA, Gulia SS, Yadav SS, Singh R, Ganguly S.** 2015. Antifungal Efficacy of *Moringa oleifera* Lam. *Am J Phyto Med Clin Ther*. **3:** 28-33.

**Zain WNIWM, Rahmat A, Othman F, Yap TYH.** 2009. Antiproliferative properties of clausine-b against cancer cell lines. *Malaysian J Med Sci*. **16**: 29-34.

**Zapata-Garrido AJ, Romo AC, Padilla FB.** 2003. Terbinafine hepatotoxicity. A casereport and review of literature. *Ann Hepatol*. **2:** 47–51.

**Zhai L, Chen M, Blom J, Theander TG, Kharazmi A.** 1999. The antileishmanial activity of novel oxygenated chalcones and their mechanism of action. *J Antimicrob Chemother*. **43:** 793-803.

**Zhang JD, Cao YB, Xu Z, Sun HH, An MM, Yan L, Chen HS, Gao PH, Wang Y, Jia XM, Jiang YY.** 2005. *In vitro* and *in vivo* antifungal activities of the eight steroid saponins from *Tribulus terrestris* L. with potent activity against fluconazole-resistant fungal. *Biol Pharm Bull*. **28**: 2211-2215.

**Zhang A, Lin G.** 2000. The first synthesis of clausenamine-A and cytotoxic activities of three biscarbazole analogues against cancer cells. *Bioorg Med Chem Lett*. **10:** 1021-1023.

**Zijlstra EE, Musa AM, Khalil EA, el-Hassan IM, el-Hassan AM.** 2003. Post-kala-azar dermal leishmaniasis. *Lancet Infect Dis*. **3:** 87–98.

**Website sources:**

**CDC (Centers for Disease Control and Prevention)**. 2017. Fungal Diseases. https://www.cdc.gov/fungal/infections/hiv-aids. html.

**Fungal Disease-Specific Research.** 2018. National Institute of Allergy and Infectious Diseases (NIAID). https://www.niaid.nih.gov/ diseases-conditions/ fungal-disease-specific-research.

**GAFFI.** 2018. Global Action Fund for Fungal Infections. *Available from*: https://www.gaffi.org.

**Special Programme for Research and Training in Tropical Diseases.** 2010. Research to support the elimination of visceral leishmaniasis—annual report 2009. TDR/BL10.10 Geneva: World Health Organization.

**WHO.** 2019. Second WHO model list of essential *in vitro* diagnostics. https:// apps. who.int/iris/handle/10665/331769.

**WHO**. 2002. WHO traditional medicine strategy 2002–2005. https://apps. who.int/iris/handle/10665/67163.

**The Times of India.** Kolkata, Friday, August 15, 2003.

**WHO.** 2009. The financial crisis and global health: report of a high-level consultation, Geneva, Switzerland, 19 January 2009. World Health Organization. https:// apps.who. int/ iris/handle/10665/70440

**WHO. 2020a.** WHO supports scientifically-proven traditional medicine, https://www. afro.who.int/ news/ who-supports-scientifically -proven-traditional-medicine

WHO 2020b. First meeting of the WHO antifungal expert group on identifying priority fungal pathogenshttps://www. who.int/ publications/ i/item/ 9789240006355.

**WHO. 2022.** https://www.who.int/news-room/fact-sheets/detail/leishmaniasis.

# APPENDIX - A

Chemicals used

| Chemicals | Company |
|---|---|
| Acetic acid (glacial) | SRL Pvt. Ltd., Mumbai, India |
| Acetic anhydride | Sigma Aldrich Chemicals Pvt. Ltd., India |
| Acetone | SRL Pvt. Ltd., Mumbai, India |
| Agar powder, Certified | HiMedia Laboratories Ltd, Mumbai, India |
| Agarose | SRL Pvt. Ltd., Mumbai, India |
| Aluminium chloride | Sigma Aldrich Chemicals Pvt. Ltd., India |
| Aluminium oxide (Neutral) for column chromatography | SRL Pvt. Ltd., Mumbai, India |
| Ammonia | SRL Pvt. Ltd., Mumbai, India |
| Ammonium oxalate | Sigma Chemical Company, St. Louis, Missouri, USA |
| Ammonium sulphate | HiMedia Laboratories Ltd., Mumbai, India |
| Ascorbic acid | SRL Pvt. Ltd., Mumbai, India |
| Barbituric acid | HiMedia Laboratories Ltd, Mumbai, India |
| Benzene | SRL Pvt. Ltd., Mumbai, India |
| Coomassie blue | Sigma Chemical Company, St. Louis, Missouri, USA |
| Crystal violet | Sigma Chemical Company, St. Louis, Missouri, USA |
| Dextrose | HiMedia Laboratories Ltd., Mumbai, India |
| Diethyl ether | SRL Pvt. Ltd., Mumbai, India |
| Dimethyl sulfoside (DMSO) | Sigma Chemical Company, St. Louis, Missouri, USA |
| 1,1-diphenyl-2-picryl-hydrazil (DPPH) | HiMedia Laboratories Ltd., Mumbai, India |
| EDTA | Sigma Chemical Company, St. Louis, Missouri, USA |

| | |
|---|---|
| Ethidium bromide | Bangalore Genei (India) Pvt. Ltd. |
| Ethyl acetate | SRL Pvt. Ltd., Mumbai, India |
| Ferric chloride | HiMedia Laboratories Ltd., Mumbai, India |
| Foetal bovine sera | Sigma Chemical Company, St. Louis, Missouri, USA |
| Formazan | Sigma Chemical Company, St. Louis, Missouri, USA |
| Gallic acid | Sigma Aldrich Chemicals Pvt. Ltd., India |
| GIEMSA | Sigma Chemical Company, St. Louis, Missouri, USA |
| Glutamine | Sigma Chemical Company, St. Louis, Missouri, USA |
| HEPES | Sigma Chemical Company, St. Louis, Missouri, USA |
| Hexane | SRL Pvt. Ltd., Mumbai, India |
| Hydrochloric acid | SRL Pvt. Ltd., Mumbai, India |
| Iodine | HiMedia Laboratories Ltd, Mumbai, India |
| Lactophenol- cotton blue | HiMedia Laboratories Ltd, Mumbai, India |
| Leishman's stain | Sigma Chemical Company, St. Louis, Missouri, USA |
| Lipophilic Sephadex | Sigma Aldrich Chemicals Pvt. Ltd., India |
| Magnesium chloride | HiMedia Laboratories Ltd., Mumbai, India |
| Magnesium ribbon | ISOCHEM, India |
| Methanol A.R. | SRL Pvt. Ltd., Mumbai, India |
| Medium-199 | GIBCOBRL, Gaithersburg, Maryland, USA |
| Molecular Biology Grade Chloroform | SRL Pvt. Ltd., Mumbai, India |
| Molybdate | Sigma Aldrich Chemicals Pvt. Ltd., India |
| MTT | Sigma Chemical Company, St. Louis, Missouri, USA |

| | |
|---|---|
| Nitro-blue tetrazolium | Sigma Chemical Company, St. Louis, Missouri, USA |
| Olive Oil | BIO VEDA ACTION RESEARCH CO., HARYANA, INDIA |
| Percoll | Pharmacia Fine Chemicals, Sweden |
| Petroleum ether | SRL Pvt. Ltd., Mumbai, India |
| Picric acid | Sigma Aldrich Chemicals Pvt. Ltd., India |
| Potassium mercuric iodide | Sigma Aldrich Chemicals Pvt. Ltd., India |
| Quercetin | HiMedia Laboratories Ltd., Mumbai, India |
| RPMI-1640 medium | GIBCO Lab, Grand Island, New York, USA |
| Riboflavin | Sigma Chemical Company, St. Louis, Missouri, USA |
| Safranin | Sigma Chemical Company, St. Louis, Missouri, USA |
| SGOT-SGPT kit | Dr. Reddy's Laboratory, Hyderabad, India |
| Silica Gel GF-254 (60-120 mesh) | SRL Pvt. Ltd., Mumbai, India |
| Silica gel (100-200 mesh) for column chromatography | SRL Pvt. Ltd., Mumbai, India |
| Silica gel (60-120 mesh) for column chromatography | SRL Pvt. Ltd., Mumbai, India |
| Sodium antimony gluconate (SAG) | Sigma Chemical Company, St. Louis, Missouri, USA |
| Sodium carbonate | HiMedia Laboratories Ltd., Mumbai, India |
| Sodium chloride | Merck Specialities Pvt. Ltd., Mumbai, India |
| Sodium stibogluconate | Gluconate Health Ltd., Sweden |
| Sodium sulphate | HiMedia Laboratories Ltd., Mumbai, India |
| Sodium thioglycolate | GIBCO Lab, Grand Island, New York, USA |
| Sucrose | SRL Pvt. Ltd., Mumbai, India |
| Sulphuric acid | SRL Pvt. Ltd., Mumbai, India |

| | |
|---|---|
| Superoxide dismutase (SOD) | Sigma Chemical Company, St. Louis, Missouri, USA |
| TEMED | Sigma Chemical Company, St. Louis, Missouri, USA |
| Trichloroacetic acid | Merck, Germany |
| Vanillin | SRL Pvt. Ltd., Mumbai, India |
| 0.22 µm pore size millipore filter | Millipore Corporation, USA |
| Gel loading buffer (6X) | Bangalore Genei (India) Pvt. Ltd. |
| TLC Silica gel 60 F254 aluminium sheets | Merck, Germany |
| 100 µg/ml streptomycin sulphate | Sigma Chemical Company, St. Louis, Missouri, USA |
| 100U/ml peniciline G-Sodium | Sigma Chemical Company, St. Louis, Missouri, USA |

# APPENDIX-B

1. **Mayer's reagent (potassium mercuric iodide)**

   **Mixture A:** 1.358 g of mercury (II) chloride dissolved 60 mL of distilled water

   **Mixture B:** 5 g of potassium iodide dissolved in 10 mL of distilled water. Mixture A was poured into Mixture B, and the final volume was made up to 100 mL with distilled water.

2. **Wagner's reagent (iodine in potassium iodide)**

   Two grams of iodine and 6 g of KI dissolved in 100 mL of distilled water.

3. **Hager's reagent (saturated picric acid solution)**

   Picric acid(1g) dissolved in 100 mL of distilled water.

4. **Molisch's reagent**

   15 g of 1-naphthol dissolved 100 mL of 95% (vol/vol) ethanol or chloroform.

5. **Benedict's reagent (qualitative)**

   **Solution A:**173 g sodium citrate and 100 g of sodium carbonate dissolved in 800 mL of distilled water, filtered, and diluted to 850 mL with distilled water.

   **Solution B:** 17.3 g of copper (II) sulphate pentahydrate in 100 mL of distilled water.

   Solution B was poured, with constant stirring, into Solution A and diluted to 1 L with distilled water.

6. **Fehling's A and B solutions**

   **Fehling's Reagent A**: 70g of copper sulphate and concentrated sulphuric acid 1ml dissolved in distilled water (200 ml) and made up to volume 1 litre with distilled water.

**Fehling's Reagent B:** 352 g of sodium potassium tartrate and 154 g sodium hydroxide dissolved in 200 ml distilled water and made up to volume 1 litre with distilled water.

7. **Folin-Ciocalteu reagent**

   Hetero-polyphospho tungstate-molybdate diluted with distilled water in 1:1 ratio

8. **Phosphate buffered saline (PBS) (0.15M)**

   **Solution A**: $Na_2HPO_4$ 4.2588 g, Distilled water 200ml.

   **Solution B**: $Na_2HPO_4$, $2H_2O$ 9.3606 g, Distilled water 400ml.

   140 ml A was mixed with 360 ml of Solution B, and 4 g of NaCl and 100 mg of KCl was added.

9. **Potassium phosphate buffer (50mM, pH7.2)**

   Solution A: 0.1 M solution of $Na_2HPO_4$ 1.42 g, Distilled water 100ml

   Solution B: 0.1M solution of $Na_2HPO_4$, $2H_2O$ 1.56 g, Distilled water 100ml

   Solution A and Solution B were separately autoclaved and stored at 40°C. 81 ml of Solution A were mixed with 19 ml of Solution B, diluted to 200 ml with distilled water to make final phosphate buffer.

10. **EDTA (0.1 mM, pH 8.0)**

    Disodium EDTA - dihydrate 18.6g

    Distilled water 100ml

    Measured amount of disodium EDTA-dihydrate was dissolved in distilled water and mixed vigorously on a magnetic stirrer. The pH was adjusted to 8.0 with NaOH. Solution was sterilized by autoclaving at 15 lbs psi pressure for 15 min at 121°C.

11. **Turk's fluid**

    Glacial acetic acid 1.5 ml, gentian violet 1% solution 1.5 ml, and distilled water 100 ml.

12. **Pyrogallol**

    0.2 mM Pyrogallol in air equilibrated, 50mM Tris-cacodylic acid buffer, and 1 mM diethylene triamine penta acetic acid, (pH 8.2)

13. **Crystal violet stain**

    **Solution A**- Crystal violet 2g, 95% Ethanol 20ml

    **Solution B**- Ammonium oxalate 0.8 g, Distilled water 80 ml

    Solution A and B was mixed for 30-60 seconds.

14. **Lugol's iodine**

    Iodine 1.0 g

    Potassium iodide 2.0 g

    Distilled water 300 ml

15. **Lactophenol Cotton-Blue (LPCB)**

    Phenol 200.0 gm
    Cotton Blue 0.5 gm
    Glycerol 400 ml
    Lactic Acid 200 ml
    Deionized Water 200 ml

16. **Safranin solution**

    Stock solution – Safranin 0 2.5 gm, 95% Ethanol 100 ml

    Working solution – Stock solution 10 ml, distilled water 90 ml

17. **1,1-diphenyl-2-picryl-hydrazil (DPPH) solution (0.2 mM)**

    3.94 mg of DPPH was dissolved in 50 ml methanol to make a solution, fresh each time.

18. **Richard's medium**

| | |
|---|---|
| $KH_2PO_4$ | 5 g |
| $KNO_3$ | 10 g |
| $FeCl_3$ | 0.02 g |
| $MgSO_4.7H_2O$ | 2.5 g |
| Sucrose | 50 g |
| Distilled water | 1000 ml |

All the constituents were taken and mixed with required distilled water by stirring and sterilized at 15 lb psi for 15 minutes.

19. **Potato Dextrose Broth (PDB)**

    Peeled Potato 40 g

    Dextrose 2g

    Distilled water 100ml

    The potato was peeled and boiled in double volume distilled water of required amount. The potato broth was filtered through cheese cloth and then required amount of dextrose was added. Finally, the medium was sterilized at 15 lb psi for 15 minutes in autoclave.

20. **Potato dextrose agar (PDA)**

    Peeled potato 40 g

    Dextrose 2 g

    Distilled water 100 ml

    Peeled potato was boiled and filtered through cheese cloth. The required amount of dextrose and 2% agar powder were added in filtrate. The agar was completely melted by heating the media before sterilization. Finally, the medium was sterilized as 15 pb psi for 15 min in autoclave.

21. **RPMI-1640**

    About 0.584 g L-glutamine, 5.95 HEPES buffer, 100 mg/ ml streptomycin and 100 IU/ml penicillin, supplemented with 10% (v/v) heat inactivated fetal calf serum (FCS) was used.

22. **Medium-199 (pH 7.4)**

    Supplemented with 10% heat inactivated foetal bovine serum, 2mM glutamine, 25mM N-2-ethansulfonic acid (HEPES), 100U/ml peniciline G-sodium, 100 µg/ml streptomycin sulphate.

23. **Ray's solid medium**

    Brain heart infusion 3.7%, Sodium chloride (w/v) 0.1%, Agar 1.5%; pH was maintained at 7.4. The ingredients were dissolved in glass-distilled water

and autoclaved. To this, 1% glucose (w/v) and 1% blood (v/v), drawn from normal rabbit heart, was added.

24. **Complex-forming reagent (For protein estimation following Lowry's method) (Lowry, 1951)**

    This reagent was preparedby mixingthree stock solutions in the proportion of 100:1:1 (vol / vol).

    Solution A: 2% (w/v) $Na_2CO_3$ in distilled water.

    Solution B: 1% (w/v) $CuSO_4 \cdot 5H_2O$ in distilled water.

    Solution C: 2% (w/v) sodium potassium tartrate in distilled water.

**25. Polyacrylamide gel electrophoresis (PAGE):**

**Stock solutions**

**Solution A**: Acrylamide stock solution (resolving gel)

For preparing acrylamide stock solution, 29.2 g of acrylamide and 0.8 gm of N N' methylene bisacrylamide were dissolved in 100 ml of warm distilled water. The stock solution was filtered with Whatmann No.1 filter paper in dark and stored in dark bottle at $4^0$C.

**Solution B**: Acrylamide stock solution (stacking gel)

For the preparation of acrylamide stock solution for the stacking gel, 10 gm acrylamide and 2.5 gm of bisacrylamide was dissolved in 100 ml warm distilled water. The stock solution was then filtered with Whatmann No.1 filter paper and stored at $4^0$C in dark bottle.

**Solution C**: Tris-HCl (resolving gel)

Tris HCL buffer was prepared by dissolving 36.6 gm of Tris base in distilled water and 0.25 ml of TEMED was added. The pH of the solution was adjusted to 8.9 with conc. HCl. The final volume of the solution was made upto 100 ml with distilled water. The solution was then stored at $4^0$C for further use.

**Solution D**: Tris HCl (stacking gel)

5.98 gm of Tris base was mixed with distilled water and 0.46 ml of TEMED was added to it. Finally the pH of the solution was adjusted to 6.7 with conc. HCl. The final volume of the solution was made upto 100 ml with distilled water and stored at $4^0$C for further use.

**Solution E**: Ammonium per sulphate

Ammonium per sulphate was prepared by dissolving 0.15 g of APS in distilled water. The final volume was made up to 10 ml. This was prepared fresh each time.

**Solution F**: Riboflavin solution

Fresh riboflavin solution was prepared by dissolving 2 mg of riboflavin in 2 M sucrose (100 ml). The solution was kept in dark bottle to protect it from light.

**Solution G**: Electrode buffer (pH 8.4)

Fresh electrode buffer was prepared by dissolving 6 g of Tris base and 28.8 g of glycine in 1000 ml distilled water.

**Preparation of gel**

**Resolving gel (10%)**
30% Acrylamide 2.55 ml
1.5 M Tris (pH 8.8) 1.95 ml
10% SDS 0.075 ml
10% APS 0.075 ml
TEMED 0.003 ml
Water 2.85 ml

**Staking gel (5%)**
30% Acrylamide 0.5 ml
1.0 M Tris (pH 6.8) 0.38 ml
10% SDS 0.030 ml
10% Ammonium persulphate 0.030 ml
TEMED 0.030 ml
Water 2.1 ml

**Protocol:**

Polyacrylamide gel electrophoresis (PAGE) was done following the method as described by Davis (1964) with some modifications. A mini slab gel (8 X 5 cm) was prepared for which, two glass plates ware thoroughly cleaned with dehydrated alcohol to remove any traces of grease and air-dried. Spacers (1.5 mm) were placed between the two glass plates on three sides and sealed with 1% agar solution. The slabs were tightly clipped to prevent any leakage of the gel solution while casting the gel. A 10% resolving gel was prepared by mixing the acrylamide stock solution (for resolving gel), Tris-HCl (pH 8.9, for resolving gel), APS (freshly prepared) and distilled water in the ratio 1:1:4:1 and carefully dispensing the mixture by a pasture pipette between the glass slabs leaving sufficient space for the stacking gel (1.5 cm). After pouring the resolving gel solution, it was immediately over layered with water and allowed to polymerize for 1.5 - 2 h. After polymerization was complete, the water over layer was poured off and the gel was washed with water to remove any unpolymerized acrylamide.

The stacking gel (4%) solution was prepared by mixing the acrylamide stock solution (for stacking gel), Tris HCl (pH 6.7, for stacking gel), riboflavin (freshly prepared) and distilled water in the ratio 2:1:1:4. The mixture was poured over the resolving gel and the comb was inserted leaving a gap of approximately one cm below the well in the stacking gel. The gel was kept under bright sunlight or fluorescent light for polymerization. After solidification of the stacking gel, the comb was removed and the wells were washed thoroughly. Following casting, the gel with the glass slabs was fitted into the electrophoresis apparatus. Chilled tris-glycine running buffer (pH 8.4) was added sufficiently in both upper and lower reservoirs of the gel apparatus. Any bubbles, trapped at the bottom of the gel, were carefully removed with a bent syringe. The gel was loaded with protein/enzyme samples and incubated at $4^0$C supplying a constant current of 2.5 mA per well continuously for 3-4 h until the dye front reached the bottom of the gel. After electrophoresis, the gel was removed from the glass plates and then the stacking gel was cut off

from the resolving gel and finally proceeded for activity staining of superoxide dismutase.

**26. Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE)**

**Stock solutions:**

**Stock solution A: Acrylamide/Bis-acrylamide**

Acrylamide 87.6 g

N`N`-bis-methylene acrylamide 2.4 g

The ingredients were dissolved in deionized water and the volume was adjusted to 300 ml. thereafter the solution was filtered and stored at 4ºC in the dark (30 days maximum).

**Stock solution B: 10% (w/v) SDS**

10 g SDS was dissolved in 90 ml water with gentle stirring and made to 100 ml with deionized water.

**Stock solution C: 10% (w/v) APS**

20 mg of APS was dissolved in 2 ml of deionized water.

**Stock solution D: 1.5M Tris-HCl, (pH 8.8)**

Tris base 27.23 g Deionized water 80 ml The ingredients were mixed and adjusted to pH 8.8 with 6N HCl. The final volume was made upto 150 ml with deionized water and store at $4^0$C.

**Stock solution E: 0.5M Tris-HCl, (pH 6.8)**

Tris base6 g

Deionized water 60 ml

These chemicals were adjusted to pH 6.8 with 6N HCl and the total volume made upto 100 ml with deionized water and store at $4^0$C.

Stock solution F: Sample buffer

Deionized water 3.55 ml

0.5M Tris-HCl (pH 6.8) 1.23 ml

Glycerol 2.5 ml 10% (w/v) SDS 2.0 ml

0.5% (w/v) Bromophenol blue 0.2 ml

Total volume 9.5 ml

Finally the solution was stored at room temperature.

ß-mercaptoethanol (50 µl) was added to 950 µl sample buffer prior to use and the sample was diluted to at least 1:2 with sample buffer and heated at $95^0$C for 4 minutes.

**Stock solution G: 10X Electrode (running) buffer, (pH 8.3)**

Tris base 30.3 g

Glycine 144.0 g

SDS 10.0 g

The ingredients were dissolved and total volume made upto 1000 ml with deionized water and stored at $4^0$C. Use: Diluted 50 ml 10X stock with 450 ml deionized water for each electrophoresis run.

**SDS-PAGE protocol:**

Protein samples were mixed with equal volume of sample buffer and heated for 5 min at $95^0$C. Gel was made according to the method of Laemmli (1970). Separating gel (10%) was used for resolving the polypeptides whereas a 4% stacking gel was used to concentrate the polypeptides. Test samples as well as pure Fe-SOD (kindly provided by Dr. Syamal Roy, IICB, Kolkata) were loaded on the gel and electrophoresis was accomplished in electrode buffer at 30mA for 4h using a Bio-Rad, Mini PROTEAN Tetra Cell Electrophoresis system. Following electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 (Sigma) for overnight and destained. The gel was finally photographed.

Brojendra Nath Seal Journal of SCIENCE

Research Article

# Phytochemical analysis of two medicinal plants of North Bengal

**Anindita Chakraborty[1*] and Bikramjit Raychaudhury[2]**

*[1]Department of Physiology, A.B.N. Seal College, Cooch Behar, 736101, West Bengal.*

*[2]Department of Physiology, Ananda Chandra College, Jalpaiguri, 735101, West Bengal.*

**E-mail: aninditajal257438@gmail.com*

**A B S T R A C T**

Medicinal plants have been used as a source of remedies since ancient times in India. Traditional medicine systems consist of large numbers of plants with medicinal and pharmacological importance. Rouvolfia Serpentina and Moringa Olifera are two such important medicinal plants traditionally used in India for their immense therapeutic properties. In this present study plants leaf extracts are screened for the presence of major phytochemical groups which are plants primary and secondary metabolites and known for their therapeutic values. These compounds are reported to have antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protective and antimicrobial activities. Qualitative analysis showed the presence of metabolites such as glycosides, phenolic compounds, alkaloids, flabinoids, tannins, saponins, steroids for both the plants ensuring their potentiality as therapeutic agent.

***Key Words**: Rouvolfia Serpentina, Moringa Olifera, medicinal plants, therapeutic properties, phytochemical*

**Publication History: Received: 13th July, 2017** **Accepted: 30th July, 2017**

## 1. INTRODUCTION

The use of herbal medicine for the treatment of diseases and infections is as old as mankind. The World Health Organization supports the use of traditional medicine provided they are proven to be efficacious and safe (WHO, 1985). In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin, lesser side effect and cost

effective qualities (Report of seminar on Herbal Drug, 2001). Medicinal plants have been used as a source of remedies against many pathological conditions since ancient times in India. Traditional medicine systems consist of large numbers of plants with medicinal and pharmacological importance and hence represent an invaluable reservoir of new bioactive molecules.

The plant kingdom is a treasure house of potential therapeutic compounds and in the recent years there has been an increasing awareness about the importance of these compounds of medicinal plants and uses them as therapeutic agents because compounds from the plants are easily available, less expensive, safe, and efficient and proven to have side effects. Recently some plants have been selected for examination and proven therapeutically effective new drugs such as anticancer drugs (Dewick 1996), antimicrobial drugs (Phillipson and Wright 1996), antihepatotoxic compounds. Some organic compounds from medicinal plants which are by primary or rather secondary metabolites provide definite physiological action on the human body. These bioactive substances include tannins, alkaloids, carbohydrates, protein, glycosides terpenoids, steroids and flavonoids (Mann 1978; Edoga et al. 2005). They are widely used in the human therapy, as well as veterinary, agriculture, scientific research and many other different areas of human interest (Vasu et al. 2009). A large number of phytochemicals also have been shown to have inhibitory effects on all types of microorganisms in vitro and in animal models (Cowan 1999).

Among The secondary plant metabolites, phenol compounds such as flavonoid, phenolic acids, tocopherols etc are most ubiquitous groups of (Singh et al. 2007). Studies revealed that plant rich in phenolic compounds possess biological properties such as antiapoptotic, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis cell proliferation activities antioxidant properties and (Brown and Rice-Evans 1998; Krings and Berger 2001; Han et al. 2007; Ali et al. 2008). In response to microbial infection plants synthesize a hydroxylated phenolic substance, flavonoids having activities against wide array of microorganisms in vitro, which may be due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie. 1996). Another metabolite, tannins interfere with protein synthesis by binding with proline rich protein. The plant extracts containing saponins, have anti inflammatory (Just et al. 1998) hemolytic (Okwu 2004) activities. Studies show that, Glycosides lower the blood pressure (Nyarko and Addy 1990). Steroids are very important compounds, having relationship with sex hormones (Okwu 2001).They also have antibacterial activities (Raquel 2007). Alkaloids have been used for centuries for their medicinal value. They have cytotoxic (Nobori et al. 1994), analgesic antispasmodic and antibacterial (Stray, 1998; Okwu and Okwu 2004) properties. The mechanism of action of some phytochemicals are shown below (Table 1)

***Table 1:*** *Mechanism of action of some phytochemicals (Tiwari et al. 2011)*

| Phytochemicals | Activity | Mechanism of action |
|---|---|---|
| Quinones | Antimicrobial | Binds to adhesins, complex with cell wall, inactivates enzymes |
| Flavonoids | Antimicrobial<br>Antidiarrhoeal | Complex with cell wall, binds to adhesins<br>Inhibits release of autocoids and prostaglandins,<br>Inhibits contractions caused by spasmogens,<br>Stimulates normalization of the deranged water transport across the mucosal cells,<br>Inhibits GI release of Acetylcholine |
| Polyphenols and Tannins | Antimicrobial<br>Antidiarrhoeal<br>Anthelmintic | Binds to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption, metal ion complexation<br>Makes intestinal mucosa more resistant and reduces secretion, stimulates normalization of deranged water transport across the mucosal cells and reduction of the intestinal transit, blocks the binding of B subunit of heat-labile enterotoxin to GM1, resulting in the suppression of heat-labile enterotoxin-induced diarrhea, astringent action<br>Increases supply of digestible proteins by animals by forming protein complexes in rumen, interferes with energy generation by uncoupling oxidative phosphorylation, causes a decrease in G.I. metabolism |
| **Phytochemicals** | **Activity** | **Mechanism of action** |
| Coumarins | Antiviral | Interaction with eukaryotic DNA |
| Terpenoids and essential oils | Antimicrobial<br>Antidiarrhoeal | Membrane disruption<br>Inhibits release of autocoids and |

| | | |
|---|---|---|
| | | Prostaglandins |
| Alkaloids | Antimicrobial<br>Antidiarrhoeal<br>Anthelmintic | Intercalates into cell wall and DNA of parasites<br>Inhibits release of autocoids and prostaglandins<br>Possess anti-oxidating effects, thus reduces nitrate generation which is useful for protein synthesis, suppresses transfer of sucrose from stomach to small intestine, diminishing the support of glucose to the helminthes, acts on CNS causing<br>Paralysis |
| Lectins and Polypeptides | Antiviral | Blocks viral fusion or adsorption, forms disulfide bridges |
| Glycosides | Antidiarrhoeal | Inhibits release of autocoids and Prostaglandins |
| Saponins | Antidiarrhoeal<br>Anticancer<br>Anthelmintic | Inhibits histamine release in vitro<br>Possesses membrane permeabilizing properties<br>Leads to vacuolization and disintegration of teguments |
| Steroids | Antidiarrhoeal | Enhance intestinal absorption of Na+ and water |

Plant products having phytomedicinal properties, can be derived from barks, leaves, flowers, roots, fruits, seeds of the plant (Criagg and David 2001). So for synthesis of complex chemical substances, knowledge of the chemical constituents of plants is essential (Mojab et al. 2003; Parekh and Chanda 2007; Parekh and Chanda 2008).

The present study investigates the fundamental scientific basis for the use of Rouvolfia Serpentina and Moringa Olifera plants leaf as therapeutic agent by defining the presence of crude phytochemical constituents .

## 2. MATERIALS AND METHODS

### 2.1. Collection of plant materials

The leaves of the plants were collected from different uncultivated farmlands of Jalpaiguri District, West Bengal. The sample leaves of two plants were identified by the authors. The leaves were air-dried and ground into uniform powder using a REMI Mixer grinder machine and kept in air tight container. The aqueous extract of each sample was

prepared by soaking 100 g of dried powdered samples in 200 ml of distilled water for 12 h. The extracts were filtered using Whatman filter paper No 42 (125 mm).

**2.2. Phytochemical screening**

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973) to identify the constituents phytochemicals.

**2.3. Test for tannins**

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

**2.4. Test for phlobatannins**

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

**2.5. Test for saponin**

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

**2.6. Test for flavonoids**

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated $H_2S0_4$. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow coloration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

**2.7. Test for steriods and terpenoids**

***Libermann Burchard's test***

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. sulphuric acid was added to the solution. Formation of brown ring at the junction and green upper layer indicates the presence of phytosterols and formation of deep red colour indicates the presence of triterpenoids.

**2.8. Test for cardiac glycosides (Keller-Killani test)**

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet

ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

### 2.9. Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

**2.9.1.** ***Mayer's Test*****:** Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow colour precipitate indicates the presence of alkaloids.

**2.9.2.** ***Wagner's Test*****:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

**2.9.3.** ***Hager's Test*****:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

### 2.10. Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

**2.10.1.** ***Molisch's Test*****:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

**2.10.2.** ***Benedict's Test*****:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

**2.10.3.** ***Fehling's Test*****:** Filtrates were hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

### 2.11. Detection of organic acids

**2.11.1.** ***Oxalic acid test*****:** To the test solution few drops of 1% $KMnO_4$ and dilute $H_2SO_4$ is added, if the colour disappears, shows the presence of organic acid.

**2.11.2.** ***Malic acid test*****:** To the test solution 2-3 drops of 40% $FeCl_3$ is added, appearance of yellowish colour proves the presence of organic acid.

## 3. RESULT

The leaf extract and the dried powder of leaf of Rouvolfia Serpentina and Moringa Olifera showed the presence of tannin, phlobatannin, saponin, flavonoids, sterols, alkaloids, carbohydrate, protein and organic acids. Both the leaf extract tested negative for the presence of triterpinoids, while only the leaf extract of Rouvolfia Serpentina tested negative for the presence of cardiac glycosides. The phytochemical constituents of Rouvolfia Serpentina and Moringa Olifera are represented in the following table (Table 2).

***Table 2**: Phytochemical constituents of Rouvolfia Serpentina and Moringa Olifera*

| Sl. no. | Phytoconstituents | Rouvolfia Serpentina | Moringa Olifera |
|---|---|---|---|
| 1. | Alkaloids | ++ | + |
| 2. | Flavonoids | + | + |
| 3. | Tannin | + | + |
| 4. | phlobatannin | ++ | ++ |
| 5. | Saponin | + | ++ |
| 6. | Steroid | + | ++ |
| 7. | Triterpinoid | - | - |
| 8. | Cardiac Glycoside | - | ++ |
| 9. | Organic acid | + | + |
| 10. | Carbohydrate | + | + |
| 11. | Protein | + | + |

## 4. DISCUSSION

The result obtained in this study suggests that the selected plants leaves are source of many phytochemical compounds and bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. They are highly recommended for further quantitative analysis of phytochemicals present and their potentiality as therapeutic agent.

## REFERENCES


Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahuand A, Bora U, Indian medicinal herbs as source of antioxidants. **Food Res Int** 41(2008)1.

Brown JE, Rice-Evans CA, Luteolin rich artichoke extract protects low density lipoprotein from oxidation in vitro. **Free Radical Res** 29(1998)247.

Cowan MM, Plant products as antimicrobial agents. **Clin Microbiol Rev** (1999) 564.

Criagg GM, David JN, Natural product drug discovery in the next millennium. **J Pharm Biol** 39(2001)8.

Del-Rio A, Obdululio BG, Casfillo J, Main FG, Ortuno A, Uses and properties of citrus flavonoids. **J Agric Food Chem** 45(1997)4505

Dewick PM, Tumor inhibition from plants: Tease and Evans (K schemp) picrre Exbeille. Global J. India, (1996)122.

Edoga HO, Okwu DE, Mbaebie BO Phytochemicals constituents of some Nigerian medicinal plants. **Afr J Biotechnol** 4(2005) 685.

Han X, Shen T, Lou H, Dietry polyphenols and their biological significance. **Int J Mol Sci** 8(2007)950.

Harborne JB, Phytochemical methods, London. Chapman and Hall, Ltd. (1973) pp. 49

Just MJ, Recio MC, Giner RM, Cueller MU, Manez S, Billia AR, Rios JL, Anti-inflammatory activity of unusual lupine saponins from Bupleurum fruticescens. **Planta Med** 64(1998)404.

Krings U, Berger RG, Antioxidant activity of roasted foods. **Food Chem** 72(2001)223.

Mann J, Secondary Metabolism. Oxford University press, London (1978) pp. 154.

Marjorie C, Plant products as antimicrobial agents. **Clincal Microbiol Rev** 12(1996)564.
Mojab F, Kamalinejad M, Ghaderi N, Vanidipour HR, Phytochemicals screening of some species of Iranian plants. **Iran J Pharm Res** 3(2003)77-82.
Nobori T, Miurak K, Wu DJ, Takabayashik LA, Carson DA, Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. **Nature** 46(1994)753.
Okwu DE, Evaluation of chemical composition of medicinal plants belonging to Euphorbiaceae. **Pak Vet J** 14(2001)160.
Okwu DE, Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. **J Sustain Agric Environ** 6(2004)30.
Okwu DE, Okw, ME, Chemical composition of Spondias mombin linn. plant parts. **J Sustain Agric Environ** 6(2004) 140.
Parekh J, Chanda S, Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. **Afr J Biomed Res** 10(2007)175.
Parekh J, Chanda S, Phytochemicals screening of some plants from western region of India. **Plant Arch** 8(2008)657.
Phillipson JD, Wright CW, Plants With antiprotozoal activity: Tease and Evans, Pharmacognosy, 14th edn. WB Saunders Company, London, (1996) pp. 612.
Raquel FE, Bacterial lipid composition and antimicrobial efficacy of cationic steroid compounds. **Biochemica et Biophysica Acta** 1768(2007)2500.
Salah N, Miller NJ, Pagange G, Tijburg L, Bolwell GP, Rice E, Polyphenolic flavonoids as scavenger of aqueous phase radicals as chai breaking antioxidant. **Arc Biochem Broph** 2(1995)339.
Singh R, Singh SK, Arora S, Evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auriculiformis A. **Cunn Fod Chem Toxicol** 45(2007)1216.
Sodipo OA, Akiniyi JA, Ogunbamosu JU, Studies on certain on certain characteristics of extracts of bark of Pansinystalia macruceras (K schemp) picrre Exbeille. **Global J Pure Appl Sci** 6(2000) 83.
Sofowara A, Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. (1993) pp. 289.
Stray F, The Natural Guide to Medicinal herbs And Plants. Tiger Books International, London, (1998) pp. 12.
Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H, Phytochemical screening and extraction: A review **.** **Internationale Pharmacutica Sciencia** 1(2011)98.
Trease GE, Evans WC, Pharmacognsy. 11th edn. Brailliar Tiridel Can. Macmillian publishers. (1989).
Vasu K, Goud JV, Suryam A, Singara Chary MA, Biomolecular and phytochemical analyses of three aquatic angiosperms. **Afr J Microbiol Res** 3(2009)418.

Environment and Ecology 37 (1A) : 263—269, January—March 2019
Website: environmentandecology.com ISSN 0970-0420

# *In vitro* Antifungal Activity of Plant Extracts against Pathogens of Clinical and Agricultural Importance and Phytochemical Analysis of the Active Compounds

**Dipanwita Saha, Anindita Chakraborty, Ramashish Kumar, Bikramjit Raychaudhury**

Received 6 September 2018; Accepted 9 October 2018; Published on 3 November 2018

**Abstract** This study was carried out with an objective to investigate the antifungal potentials of 14 plants known for medicinal properties against the human pathogen, *Candida albicans* and the tea pathogen, *L. theobromae.* Soxhlet extracts prepared from leaves in various organic solvents were first screened for their antifungal activity by agar diffusion method. While all tested plants showed antifungal activity, evident from distinct inhibition zones on PDA plates, extracts from *Clausena excavata, Ocimum sanctum, Piper betle, Polyalthia longifolia* and *Xanthium strumarium* exhibited higher activities and were selected for phytochemical analysis. *L. theobromae* was found to be more susceptible than *C. albicans.* Bioautography with the test pathogens revealed the presence of antifungal compounds which appeared as clear zones of inhibition against fungus growth on developed TLC plates. *X. strumarium* and *C. excavata* showed two antifungal zones each while each of the other 3 extracts produced single antifungal zone. Application of spray reagents on TLC revealed the chemical nature of all active compounds. Both compounds from *X. strumarium* were sesquiterpenes and those from *C. excavata* were furano-coumarins. Bioactive compounds from *O. sanctum, P. longifolia* and *P. betle* were found to be monoterpene, diterpene and phenolic respectively. The results form the basis for further characterization and development of newer fungicidal compounds.

**Keywords** Antifungal, Bioautography, *Clausena excavata, X. strumarium, P. betle.*

Dipanwita Saha*, Anindita Chakraborty, Ramashish Kumar
Department of Biotechnology, University of North Bengal, Siliguri 734013, India

Bikramjit Raychaudhury
Department of Physiology, Ananda Chandra College, Jalpaiguri, WB, India
e-mail : dsahanbu@yahoo.com
*Corresponding author

## Introduction

Plant extracts has been traditionally used in India for treating various ailments and forms part of the cultural practice of this country. Scientific reports indicate that plants are an immense reservoir of valuable source of molecules with strong antimicrobial potential and considered as the prime source for the discovery of novel drugs. Different types of compounds produced by plants, particularly the secondary metabolites, may possess antifungal activity and some have successfully been utilized for clinical application (Atanasov

et al. 2015). In addition,many such antifungal compounds are now used in agriculture because synthetic chemical fungicides are unpopular for their residual toxicity, non targeted environmental impacts and direct or indirect effects on animal health system (Saha et al. 2012). Plant products are safer and eco-friendly in comparison to synthetic fungicides, as they have no deleterious effect on environment and on non target organisms in nature.

In recent years, *Candida* infections pose a serious threat to immunocompromised patients, especially those tested HIV positive and those who are receiving immunosuppressive drugs (Sanglard 2016, Campoy and Adrio 2017). Fungal infections lead to 1.5 to 2 million deaths per year, which is higher than either malaria or tuberculosis related deaths (Denning and Bromley 2015). Moreover, increasing resistances towards synthetic antifungal drugs are being reported (Cowen et al. 2014). *Lasiodiplodia theobromae*, a pathogen causing several crop diseases including diplodia disease in tea has also exhibited resistance against the fungicide thiopahanate methyl (Al-Jabri et al. 2017). Since tea is an important contributor to the economy of North East India, it is essential to look for effective alternatives for controlling diseases in tea plants.

The present study reports the antifungal activity of 14 plant extracts against the human pathogen, *Candida albicans* and the tea pathogen, *L.theobromae*. The chemical nature of the antifungal compounds present in the botanical extracts was determined following separation by thin layer chromatography and bioautography.

## Materials and Methods

### Selection and collection of plant materials

The plant materials were selected on the basis of literature reports of their traditional ethnomedicinal uses and bioactivity as well as availability in sub-Himalayan region of West Bengal. Altogether 14 plant

**Table 1.** List of plants used in this study.

| Name of plants | Local/English name | Family | Traditional application and biological activity |
|---|---|---|---|
| *Bidens pilosa* Linn. | Spanish needle | Asteraceae | Leaf extract used for treatment of cough, laryngitis, headache, conjunctivitis, rheumatism, infection, digestive and stomach disorder including peptic ulcer |
| *Clausena excavata* Burm. f. | Agnijal | Rutaceae | Used in cold, malaria, abdominal pain, snake-bite, preliminary stage of AIDS and dermatopathy |
| *Datura stramonium* L. | Datura | Solanaceae | Smoke of leaves is used for asthma, causes sleepiness. Roots are good for tooth-ache |
| *Datura innoxia* Mill. | Safed dhatura, Indian-apple | Solanaceae | Leaves are used as repellent and vermicide, used in asthma, wound, malaria and Leishmaniasis. Seed are grind and cooked in mustard oil to cure scabies |
| *Emblica officinialis* Gaertn. | Amla | Phyllanthaceae | Fruits used to promote longevity, enhance digestion, strengthen heart, purify blood, stimulate hair growth and enhance intellect. Used against constipation, fever and cough |
| *Eucalyptus globules* Labill. | Eucalyptus | Myrtaceae | Leaf essential oil used as antiseptic, against coughs and colds, sore throats and other infections. Used as mouthwash toothpaste. Leaf extracts used in anti-bacterial, antioxidant and anti-inflammation deodorant |
| *Lantana camara L*. var aculeate Moldenke | *Raimuniya*, Guye genda | Verbenaceae | Used in bronchitis, stomach problems, rheumatism and to clean teeth |
| *Leonurus sibiricus* L. | Guma & Raktadron | Lamiaceae | Anti-inflammatory and anti-diarrhoea. Leaf extract used in hemorrhage, weakness |
| *Measa indica* (Roxb.) A. DC. | Ramjani | Primulaceae | Leaves used as an agent for clearing the throat/vocal cord for producing a melodious sound |

**Table 1**. Continued.

| Name of plants | Local/English name | Family | Traditional application and biological activity |
|---|---|---|---|
| *Ocimum sanctum* L. | Tulsi | Lamiaceae | Leaf juice with honey is given for 3-7 days for cough and cold. 1 : 1 ratio of tulsi leaf and neem leaf paste is very effective for diabetes |
| *Polyalthia longifolia* var *pendula* | Ashok tree | Annonaceae | Powder of stem bark mixed with curd and sugar and mixture is given orally thrice a day to cure diarrhoea. Stem bark is dried, powdered and given orally in the treatment of gout |
| *Piper betle* L. | Paan | Piperaceae | Leaf paste with *Acacia catchu* bark paste massaged on the skin of children in maggots. Herbal dye, antipyretic, antioxidant, anticancer, antiulcer, anti-inflammatory, pain reliever and immunomodulating |
| *Syzygium cumini* (L.) Skeels | *Jamun* | Myrtaceae | Hypoglycaemic, diuretics, analgesic, anti-inflammatory, antiplaque, antimicrobial antidiarrhoel, antioxidant and gastroprotective |
| *Xanthium strumarium* L. | Chotagokhru | Asteraceae | Dry fruits kept on dried stem of *Calotropis procera* are burnt and the smoke is inhaled |

species were studied for their biological activity and phytochemical analysis. Table 1 shows a list of the plants along with their local names, families and traditional uses (Rastogi and Mehrotra 1995, Chatterjee and Pakrashi 1997). Most of the plant materials were coolected from local areas within and outside campus of University of North Bengal. Some of the plants were collected from forest areas of Sukna located in the Terai region of the Eastern Himalayas. Fresh disease free leaves of different plant species were collected. Voucher specimen of each species was deposited in the herbarium of Department of Botany, University of North Bengal.

## Fungal pathogens

Two pathogens of clinical and agricultural importance were used in this study. A virulent strain of *Lasiodiplodia theobromae* (ITCC 5446.02) was earlier isolated in the laboratory from young tea plants showing diplodia disease from a nursery in the Darjeeling district of West Bengal and its identity was authenticated by IARI, New Delhi. *Candida albicans* (MTCC 183) which is an opportunistic human pathogen causes candidiasis especially in immunocompromised patients. This strain was obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India.

## Soxhlet extraction

Fresh leaves of fourteen different plant species (Table 1) were thoroughly washed with distilled water and shade dried at room temperature for 5 to 10 days. The dried leaves (120 g) of each plant were ground to moderately fine powder (1mm) and extracted in a soxhlet apparatus using appropriate solvents (Table 2), for fifteen h at 35—45°C. The extract was concentrated to dryness under vacuum in a rotary evaporator (Eyela, Japan). The residue (15 g) obtained as a gummy solid mass was dissolved in proper solvent at 3 different concentrations (100 mg/ml, 10 mg/ml and 1 mg/ml) and used for further studies.

## Agar cup bioassay

The plant extracts were screened for antifungal activity by agar cup diffusion method. Potato dextrose agar (PDA) medium was autoclaved at121°C for 15 min, cooled to 45 °C and 1ml of pure cell suspension or spore suspension ($10^6$ /ml) or of the test pathogen was mixed with 19 ml of molten medium and poured into sterile petriplates of 9 cm diameter. Agar cups were prepared with sterile cork-borer (4 mm diameter) in the PDA plates after solidification of the medium seeded with spores of the test fungi. Plant extracts (50 µl) were introduced into each well and the plates were incubated for 48-72 h at 28°C. The antifungal activity

**Table 2.** Screening of antifungal potential of crude soxhlet extracts of leaves from different plant species against *Candida albicans* and *Lasiodiplodia theobromae* by agar diffusion assay.* The inhibition zone includes the diameter of the agar cup (6 mm).

| Name of plants | Solvent for extraction | Diameter of inhibition zone (mm) obtained at different concentrations of leaf extracts (mg/ml)* *C. albicans* 100 | *C. albicans* 10 | *C. albicans* 1 | *L. theobromae* 100 | *L. theobromae* 10 | *L. theobromae* 1 |
|---|---|---|---|---|---|---|---|
| *Bidens pilosa* | Ethyl acetate | 21.3 ± 3.0 | 15.0 ± 1.7 | 9.3 ± 1.5 | 25.0 ± 2.6 | 16.6 ± 2.1 | 11.3 ± 2.5 |
| *Clausena excavata* | Ethyl acetate | 24.3 ± 3.5 | 17.3 ± 2.5 | 7.6 ± 0.6 | 34.6 ± 3.2 | 22.3 ± 3.0 | 14.3 ± 2.1 |
| *Datura stramonium* | Benzene | 17.0 ± 4.5 | 10.6 ± 1.2 | 0 | 26.3 ± 2.5 | 15.0 ± 2.6 | 0 |
| *Datura innoxia* | Ethanol | 18.6 ± 3.8 | 12.0 ± 2.6 | 8.3 ± 0.6 | 28.6 ± 3.2 | 18.3 ± 2.3 | 8.6 ± 1.1 |
| *Emblica officinalis* | Ethyl acetate | 13.3 ± 2.1 | 7.6 ± 0.5 | 0 | 17.6 ± 3.8 | 13.3 ± 2.1 | 0 |
| *Eucalyptus globulus* | Ethanol | 18.3 ± 1.5 | 9.3 ± 2.1 | 0 | 21.6 ± 3.2 | 14.6 ± 2.1 | 9.0 ± 1.0 |
| *Lantana camara* | Ethanol | 11.6 ± 1.1 | 0 | 0 | 15.6 ± 2.5 | 10.6 ± 1.5 | 0 |
| *Leonurus sibiricus* | Ethanol | 15.6 ± 2.1 | 10.6 ± 0.6 | 0 | 20.3 ± 2.3 | 14.0 ± 3.6 | 7.3 ± 0.6 |
| *Maesa indica* | Dichloromethane | 18.6 ± 2.9 | 14.3 ± 3.0 | 8.3 ± 0.6 | 25.6 ± 2.5 | 15.6 ± 2.5 | 10.3 ± 2.1 |
| *Ocimum sanctum* | Ethyl acetate | 22.0 ± 2.6 | 13.6 ± 2.1 | 9.6 ± 1.5 | 26.3 ± 1.5 | 18.3 ± 4.6 | 11.6 ± 3.0 |
| *Polyalthia longifolia* | Ethyl acetate | 31.3 ± 2.5 | 16.3 ± 2.3 | 11.0 ± 2.6 | 28.3 ± 2.3 | 23.3 ± 3.2 | 15.6 ± 3.0 |
| *Piper betle* | Dichloromethane | 29.6 ± 3.2 | 17.3 ± 1.5 | 12.6 ± 1.5 | 36.3 ± 2.9 | 24.6 ± 4.0 | 14.3 ± 1.5 |
| *Syzygium cumini* | Ethyl acetate | 15.3 ± 3.0 | 9.6 ± 1.5 | 0 | 22.3 ± 3.8 | 13.6 ± 0.6 | 11.0 ± 1.0 |
| *Xanthium strumarium* | Benzene | 24.6 ± 3.0 | 18.3 ± 2.1 | 9.6 ± 1.5 | 35.3 ± 3.5 | 24.3 ± 2.5 | 15.6 ± 3.0 |

was evaluated by measuring zones of inhibition of fungal growth around the plant extracts. Complete antifungal assay was carried out under strict aseptic conditions. The zones of inhibition were measured in mm and the experiment was carried out in triplicate. The average of 3 replications and standard deviation were computed by MS Excel 2007.

## Thin layer chromatography

Thin layer chromatography was used both for detection of antifungal compounds and their phytochemical analysis. Antifungal activities were tested by bioautography following the method of Kumar et al. (2012). Phytochemical analysis was performed using ultraviolet light and different spray reagents (Wagner and Bladt 1996). Precoated silica gel 60 F254 aluminium TLC plate (Merck, India) was activated by heating at 70°C for 45 minutes prior to sample-loading. Each concentrated extract (10 mg/ml) was loaded on the activated TLC plate at 2 different spots (20 µl each) 2 cm apart and developed either in hexane : ethyl acetate : methanol (60 : 40 : l v/v) or in hexane : ethyl acetate (70 : 30 or 80 : 20 v/v) as solvent. The plates were air-dried until the solvent evaporated completely and subsequently cut symmetrically into 2 parts to separate the 2 developed chromatograms. These were used independently for phytochemical analysis and bioautography and the results were compared in order to determine the nature of antifungal compound.

## Bioautography

For bioautography with *L. theobromae*, spore suspension ($10^6$ spores/ml) was prepared from 7 d old culture in Richard's medium and sprayed with an atomizer on dried TLC plates (Kumar et al. 2012). The plates were incubated in a humid chamber at 28°C for 2-5 days. For *C. albicans* developed chromatograms were placed in sterile petri plates. Molten PDA medium was mixed with phenol red (0.02%) and an inoculum of cell suspension of *Candida albicans*, at tolerable temperature. The mixture was poured evenly over developed TLC plates, covered with lid and incubated at 28°C for 24 h. Inhibition zones, which appeared as clear spots on a background of fungal growth, indicated the presence of antifungal compounds. $R_f$ values of the inhibition zones and the zone diameters swere noted.

## Phytochemical analysis

For phytochemical analysis, the developed chromatogram was viewed under UV light (254 and 365 nm) and sprayed with vanillin-sulfuric acid, anisaldehyde-sulfuric acid or Folin ciocalteu's reagent. The color of the developed spots, if any, was noted and

**Table 3.** Antifungal activity of crude soxhlet extracts of leaves from potential plant species against *Candida albicans* and *Lasiodiplodia theobromae* assessed by bioauto graphy technique and phytochemical detection of antifungal compounds.

| Plants | No. of zones | $R_f$ | Antifungal activity Zone of inhibition (mm) *C. albicans* | *L. theobromae* | Chemical nature of active compound |
|---|---|---|---|---|---|
| *C. excavata* | 2 | 0.39 | 18 | 25 | Furano-coumarin |
| | | 0.30 | 15 | 22 | Furano-coumarin |
| *O. sanctum* | 1 | 0.85 | 10 | 15 | Monoterpene |
| *P. betle* | 1 | 0.64 | 30 | 38 | Phenolic |
| *P. longifolia* | 1 | 0.73 | 15 | 27 | Diterpene |
| *X. strumarium* | 2 | 0.56 | 32 | 38 | Sesquiterpene |
| | | 0.25 | 24 | 32 | Sesquiterpene |

the $R_f$ was matched with that of the active compound. The result of chemical analysis was determined based on color of the spots (Wagner and Bladt 1996, Harborne 2005).

## Results and Discussion

### Screening of botanicals for antifungal activity

Agar cup bioassay showed that all 14 plants were active against both the tested fungal pathogens (Table 2). But overall *L. theobromae* was found to be more susceptible than *C. albicans. Piper betle* and *Xanthium struaium* was found to be the most active plants as it produced largest inhibition zones of 36.3 mm and 35.3 mm respectively at 100 mg/ml against *L. theobromae.* Other plants showing strong antifungal activity were *Polyalthia longifolia, Clausena excavata* and *Ocimum sanctum*. Besides *Datura innoxia* and *D. stramonium* also showed good antifungal activity and 100mg/ml which however reduced greatly at 1 mg/ml. *Emblica officinalis* and *Lantana camara* were less effective. Reports of antifungal activity of *Maesa indica* and *Leonurus sibiricus* is extremely rare (Yashoda et al. 2014). Considering the overall performances of the plant species, 5 plants, viz. *P. betle, O.sanctum, P.longifolia, C. excavata* and *X. strumarium* were selected for further phytochemical analysis.

### Activity monitoring and phytochemical analysis of active compounds

Bioautography of the concentrated crude extracts prepared from *C.excavata, X. strumarium,P. longifolia, P.betle* and *O. sanctum* revealed antifungal activity against *C. albicans* and *L. theobromae* (Table 3). The occurrence of antifungal components was evident by the presence of clear zones of inhibition on TLC plates. *X. strumarium* and *C. excavata* showed 2 antifungal zones each while the other 3 plant extracts produced single antifungal zone each. The largest inhibition zone of 38 mm was produced by *X.strumarium* ($R_f$ 0.56) and *P. betle* ($R_f$ 0.64) extracts against *L.theobromae. X. strumarium* showed 2 antifungal compounds both of which produced green fluorescence under $UV_{254}$. The compounds produced reddish brown spots when sprayed with anisaldehyde-sulfuric acid and brown spots with vanillin-sulfuric acid which indicated presence of sesquiterpene derivatives. *X. strumarium* has been reported to contain an antimicrobial sesquiterpine lactone named xanthatin (Saha et al.2012). Another sesquiterpine lactone, deacetyl xanthumin, has been reported but this also structurally resembles xanthatin (Kim et al. 2002). Occurrence of a second antifungal compound in our extract indicates that this plant contains yet undetected bioactive compounds which warrants further study.

The single antifungal compound of *P. betel* produced green fluorescence under $UV_{254}$ and deep blue and brown spots when sprayed with Folin ciocaltu's and vanillin-sulfuric acid respectively indicating phenolic compound. Row and Ho (2009) studied the chemical compositions of the crude oil by GC/MS analysis and identified 36 compounds including eugenol (36.2%) and chavibetol acetate (16.9%) both of which are antifungal phenolic compounds or their derivatives (Kumar et al. 2010b). In this study, only one large antifungal zone was observed possibly because the compounds failed to separate under the current experimental conditions.

*P. longifolia* (27 mm) and *C. excavata* (25 mm) also exhibited large inhibition zones against *L. theobromae*, however, zone diameters produced by *O. sanctum* (15 mm) were much lower than other

extracts. The two antifungal compounds from *C. excavata* showed intense quenching under $UV_{254}$ but fluoresced with a bright blue color under $UV_{365.}$ Both produced blackish blue spot upon spraying with anisaldehyde-sulfuric acid indicating presence of furano-coumarins. Literature review has revealed that root of *C. excavata* have diverse group of coumarins with broad range of biological activities (Wang et al. 2008). However, till date, very few studies have been done concerning the antifungal activity of phytochemicals of *C. excavata* leaves. We earlier reported a new compound excavarin A (Kumar et al. 2012) from dichloromethane extract. Characterization of this second compound with antifungal activity in the ethyl acetate extract is in progress.

The antifungal compound from *P.longifolia* appeared as a single blue spot on TLC plates upon spraying with anisaldehyde reagent and heating at 110°C which indicated the presence of diterpene. The occurrence of clerodane and halimane diterpenes in *P. longifolia* has been reported by several authors (Katkar et al. 2010, Bhattacharya et al. 2015). The antifungal compound from *O. sanctum* produced brown spots when sprayed with vanillin sulfuric acid indicating monoterpene derivative. Essential oils from *O.sanctum* leaves have been found to posses antifungal properties (Kumar et al. 2010 a). Eugenol, which is also a monoterpene has been reported to be the major compound (43%) in essential oil extract by GC-MS analysis (Devendran and Balasubramanian 2011). Thus it is most probable that the antifungal compound detected in this study is eugenol, but further studies are necessary to ascertain this.

## Conclusion

The studies showed that extracts from several plants, especially, *X. strumarium* and *P. betle* has strong antifungal activity against *L. theobromae* and *C. albicans.* The phytochemical analysis coupled with bioautography revealed occurrences of several types of antifungal compounds which may form basis for further characterization and development of newer fungicidal compounds.

**References**


Al-Jabri MK, Al-Shaili M, Al-Hashmi M, Nasehi A, Al-Mahmooli IH, Al-Sadi AM (2017) Characterization and evaluation of fungicide resistance among *Lasiodiplodia theobromae* isolates associated with mango dieback in oman. J Pl Pathol 99 : 753—759.

Atanasov, AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Rollinger JM, Schuster D, Breuss JM, Bochkov V, Mihovilovic MD, Kopp B, Bauer R, Dirsch VM, Stuppner H (2015) Discovery and resupply of pharmacologically active plant-derived natural products : A review. Biotechnol Adv 33 : 1582—1614.

Bhattacharya AK, Chand HR, John J, Deshpande MV (2015) Clerodane type diterpene as a novel antifungal agent from *Polyalthia longifolia* var *pendula.* Eur J Med Chem 94 : 1—7.

Chatterjee A, Pakrashi SC (1997) The Treatise of Indian Medicinal Plants, Vol 1—6. New Delhi, National Institute for Science Communication.

Campoy S, Adrio JL (2017) Antifungals. Biochem Pharmacol 133 : 86—96.

Cowen LE, Sanglard D, Howard SJ, Rogers PD, Perlin DS (2014) Mechanisms of antifungal drug resistance. Cold Spring Harb : Perspect. Med. doi : 10.1101/cshperspect.a019752

Denning DW, Bromley MJ (2015) How to bolster the antifungal pipeline . Science 347 : 1414-1416.

Devendran G, Balasubramanian U (2011) Qualitative phytochemical screening and GC-MS analysis of *Ocimum sanctum* L. leaves. Asian J Pl Sci Res 1 : 44—48.

Harborne JB (2005) Phytochemical methods, A guide to modern techniques of plant analysis. 3rd edn. Springer (India) Private Limited, New Delhi.

Katkar KV, Suthar AC, Chauhan VS (2010) The chemistry, pharmacologic and therapeutic applications of *Polyalthia longifolia*. Pharmacogn Rev 4 : 62—68.

Kim DK, Shim CK, Bae DW, Kawk YS, Yang MS, Kim HK (2002) Identification and biological characteristics of an antifungal compound extracted from cocklebur (*Xanthium strumarium* ) against *Phytophthora drechsleri*. Pl Pathol J 18 : 288—292.

Kumar N, Mishra P, Dube A, Bhattacharya S, Dikshit M, Rande S (2010a) *Piper betle* Linn. a maligned Pan-Asiatic plant with an array of pharmacological activities and prospects for drug discovery. Curr Sci 99 : 922—932.

Kumar A, Shukla R, Singh P, Dubey NK (2010b) Chemical composition, antifungal and antiaflatoxigenic activities of *Ocimum sanctum* L. essential oil and its safety assessment as plant based antimicrobial. Food Chem Toxicol 48 : 539—543.

Kumar R, Saha A, Saha D (2012) A new antifungal coumarin from *Clausena excavate*. Fitoterapia 83 : 230—233.

Rastogi RP, Mehrotra BN (1995) Compendium of Indian Medicinal Plants, Vol. 4. New Delhi, CDRI, Lucknow and Publications & Information Directorate.

Row LCM, Ho JC (2009) The antimicrobial activity, mosquito larvicidal activity, antioxidant property and tyrosinase

inhibition of *Piper betle*. J Chinese Chem Soc 56 : 653—658.

Saha D, Kumar R, Ghosh S, Kumari M, Saha A (2012) Control of foliar diseases of tea with *Xanthium strumarium* leaf extract. Ind Crop Prod 37 : 376—382.

Sanglard D (2016) Emerging threats in antifungal-resistant fungal pathogens. Front Med 3 : 11.

Wagner H, Bladt S (1996) Plant Drug Analysis. Springer-Verlag Berlin Heidelberg.

Wang YS, He HP, Yang JH, Di YT, Hao XJ (2008) New monoterpenoid coumarins from *Clausena anisum-olens*. Molecules 13 : 931—937.

Yashoda K, Manasa M, Vivek MN, Prashith Kekuda TR (2014) Inhibitory effect of some plants of Western Ghats of Karnataka against *Colletotrichum capsici*. Sci Technol Art Res J 3 : 76—82.

*ejpmr, 2017,4(11), 336-340*

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# IDENTIFICATION AND PURIFICATION OF FE CONTAINING SUPEROXIDE DISMUTASE FROM LEISHMANIA DONOVANI

**Bikramjit Raychaudhury*[1], R. Jyoti[1], Kakuli Chakraborty[1], Anindita Chakraborty[2], Moushree Palroy[1] and Rajen Haldar[3]**

[1]Department of Physiology, Ananda Chandra College, Jalpaiguri 735101.
[2]Department of Physiology, ABN Seal College, Coochbehar 736101.
[3]Department of Physiology, University Colleges of Science and Technology, University of Calcutta, Kolkata, India.

***Corresponding Author: Bikramjit Raychaudhury**

Department of Physiology, Ananda Chandra College, Jalpaiguri 735101.

**Article Received on 22/08/2017** **Article Revised on 12/09/2017** **Article Accepted on 03/10/2017**

## ABSTRACT

Leishmaniasis is a parasitic disease which infects as many as 400,000 people per year. Because the infective agent- a protozoan-inhabits phagolysosomes in host macrophages, the parasites are partially protected from chemotherapeutic agents. Thus, treated patients often relapse or experience toxic reactions to the drugs. In order to develop new leishmanicidal agents, studies have been conducted to understand the interactions of the parasite with the macrophage. Ordinarily, microorganisms which are taken up by macrophages are destroyed by oxygen-dependent and oxygen-independent antimicrobial systems. The oxygen-dependent antimicrobial activity of macrophages is dependent on the generation of superoxide ($O_2^-$) by the one-electron reduction of molecular oxygen. This $O_2^-$ can then undergo a series of reactions to produce hydrogen peroxide ($H_2O_2$), hydroxyl radicals ($^{\cdot}OH$), and perhaps, singlet oxygen ($^1O_2$), which may be responsible for destroying the ingested microorganism. Some microorganisms, such as Toxoplasma gondii, block the generation of superoxide by macrophages in order to survive phagocytosis. It is found that this was not the case for Leishmania. The uptake of Leishmania tropica promastigotes by mouse peritoneal macrophages was accompanied by the generation of high levels of superoxide and other activated forms of oxygen. The possibility that parasites might survive phagocytosis by efficient enzymatic decomposition of $H_2O_2$ and $O_2^-$ has also been investigated. The parasites were found to lack or contain low levels of enzymes capable of decomposing $H_2O_2$ (glutathione peroxidase and catalase) but to contain relatively high levels of superoxide dismutase, which degrades $O_2^-$. Evidence was obtained that the leishmanial superoxide dismutase was substantially different from the superoxide dismutase found in its host and therefore made a potential target for the design of parasite-specific enzyme inhibitors. The leishmanial superoxide dismutase activity was found to be insensitive to inhibition by cyanide, but sensitive to inhibition by azide and peroxide, properties which suggest an Fe containing superoxide dismutase. In contrast, the mammalian host has only Cu/Zn-containing and Mn-containing superoxide dismutases. In order to further characterize the leishmanial superoxide dismutase, we have isolated superoxide dismutase from *Leishmania donovani*.

**KEYWORDS:** Leishmania donovani, Superoxide dismutase, Antioxidant.

## INTRODUCTION

The superoxide radical is an intermediate reduction product of oxygen produced by a variety of biological reactions. The superoxide radical ($O_2$) and other reactive derivatives have received recent attention as agents of oxygen toxicity in cells. Most organisms, therefore, have defense systems, such as metallo-enzymes, to protect themselves from toxic oxygen species. Metallo-enzymes that catalyze the disproportionation of superoxide free radicals ($O_2$) to hydrogen peroxide ($H_2O_2$) and oxygen ($O_2$), are known as superoxide dismutases (SODs). SODs play an important role in the protection of cells from the oxidative damage of superoxide radicals. Cell damage may also be due to the superoxide itself or, indirectly, even more reactive oxygen species, such as hydroxyl radicals ($^{\cdot}OH$), formation of which, via the Fenton reaction, is favoured by excess superoxide[1] SODs have subunit molecular weights ranging from 16 kDa to 26 kDa and are divided into three classes on the basis of their active site metals: copper and zinc (Cu/Zn-SOD), manganese (Mn-SOD), and iron (Fe-SOD). Cu/Zn-SOD is found widely in the cytoplasm and certainly in the mitochondrial inter-membrane space of the eukaryotic cells and chloroplasts of plants. Mn-SOD is located in prokaryotes and in the mitochondria of eukaryotes[2] while Fe-SOD has been found in bacteria, blue-green algae and protozoa[3] Recent reports also indicated that the enzyme was present in higher land plants[4] In addition, Ni-SOD has been isolated from some microorganisms.[5]

NADPH oxidase, also referred to as phox, is expressed in professional phagocytes such as neutrophils and macrophages. It catalyzes the production of large amounts of $O_2^{\cdot-}$ (~10 mM inside the phagosome) upon activation. The neutrophil is particularly efficient and produces levels 2-3 times higher than those of macrophages. The important role of phox to the immune defense in exemplified by the fact that a defective enzyme results in chronic granulomatous disease (CGD)**[6].** People with CGD have a genetic deficiency of phox components and suffers from recurrent infections. There are also other cellular sources of $O_2$, including xanthine oxidoreductase and homologues of the gp91phox subunit of phox. In addition, the mitochondria and certain metabolic pathways generate $O_2$.

Superoxide dismutases (SODs) represent a family of metalloenzymes, found in organisms ranging from bacteria to humans.[7] SODs exist in several different isoforms that differ in their structure and prosthetic ion/ions but all isoforms catalyze the dismutation of $O_2^-$ to hydrogen peroxide ($H_2O_2$) and oxygen ($O_2$). Iron-SOD (Fe-SOD) and Manganese-SOD (Mn-SOD) are located in the cytoplasm of prokaryotes whereas Cu/Zn-SOD is located in the periplasm of gram-negative bacteria, anchored to the outer membrane, or secreted[8] Thus, Cu/Zn-SOD in bacteria has the potential to protect against reactive oxygen species (ROS) generated from external sources. Accordingly, it has also been shown to be a virulence mechanism of many bacteria. The amino acid sequences of Cu/Zn-SOD from different bacterial species show extensive variation[9] and is predicted to affect the architecture of the active site channel and subunit assembly and, hence, enzyme activity. Thus, the SOD activity may vary substantially between bacterial species.

## MATERIALS AND METHODS

### Reagents

Brain heart infusion broth was obtained from Acumedia Manufactures, Baltimore, Maryland, USA. Bactoagar was procured from DIFCO laboratories, Detroit, Michigan, USA. The culture media Medium 199, fetal bovine serum, penicillin-streptomycine, HEPES buffer and L-glutamine were purchased from GIBCO Laboratories, Grand Island, New York, USA. Alkaline phosphatase conjugated anti-rabbit and mice IgG (whole molecule) were from Sigma Immunochemicals. TRITC and FITC coupled anti rabbit and mice IgG were obtained from Bangalore Genei, India. Other reagents were of highest purity available from Sigma Chemical Company, St. Louis, Missouri, USA.

DEAE-52 was purchased from Whatman, and Sephadex G-75 from Amersham Pharmacia Biotech Corporation (Sweden). Molecular weight markers for gel electrophoresis were obtained Amersham Pharmacia Biotech Corporation (Sweden). The other chemicals were analytical reagents.

### Parasite

*Leishmania donovani* strain MHOM/IN/AG/83 was obtained from kala-azar patient[10] and maintained by intracardial passage in every 8 weeks in Syrian golden hamster. Promastigotes were obtained by transforming amastigotes isolated from infected spleen[11] and maintained in medium -199 supplemented with 10% fetal calf serum *in vitro*. The strain was also maintained at 22° C in modified Rays medium.[12]

### Purification of Fe-SOD from *Leishmania donovani*

### Preparation of cell lysate

A total of 25 gm Leishmania cells were taken at this stage for purification of Fe-SOD. Cells were dissolved in x 2 volume ice-cold buffer A containing 50 mM potassium phosphate, 10mM TES, 1mM EDTA, 0.5 mM phenylmethylsulfonyl (in 0.1% ethanol) fluoride and 0.25 mM leupeptin at pH 7.6 and were sonicated by giving 5-6 strokes of 20 sec in an MSE sonicator at 4°C on a setting of 6 and centrifuged at 100,000g for 60 min in a Sorvall centrifuge. The supernatant cell free extract obtained was then used in subsequent steps of purification. .

### $(NH4)_2SO_4$ precipitation

The cell-free extract was treated with $(NH4)_2SO_4$ in three steps. First, solid $(NH4)_2SO_4$ was added to the extract to 30% saturation at 4ºC, then the mixture was stirred for 15 min at 4ºC, and left at 4ºC for 60 min. The precipitate was removed by centrifugation at 9,000 rpm for 10 minutes. In the second step, the supernatant was treated with solid $(NH4)_2SO_4$ to 60% saturation at 4ºC, stirred for 15 min at 4ºC, and left at 4ºC for 60 min. The precipitate was removed by centrifugation at 9,000 rpm for 10 minutes. In the third step, the supernatant was treated with solid $(NH4)_2SO_4$ to 90% saturation at 4ºC, stirred for 15 min at 4ºC, and left at 4ºC for 60 min. The precipitate with SOD activity was centrifuged at 9000 rpm for 10 min (Hettich, Universal 30RF centrifuge) and then dissolved in a minimal volume of 20 mM potassium phosphate (pH 7.6 buffer B) and dialyzed overnight at 4ºC against the same buffer. The SOD solution was concentrated to 0.5 ml by a Amicon PM – 30 (30,000-molecular weight cut off).

### Ion-exchange Chromatography

The concentrated enzyme solution was loaded on to a DEAE-52 column (2.6 cm x 60 cm) and equilibrated with buffer B for 5 h at 0.25 ml / min. The column was eluted with a 0–500 mM NaCl linear gradient and fractions of 3 ml were collected. The SOD active fractions were pooled, concentrated by Amicon PM–30 (30,000-molecular weight cut off) at 4ºC to 10 ml and dialyzed against 10 mM phosphate buffer (buffer C, pH 7.6) for 24 h (4ºC).

### Gel Chromatography

The concentrated enzyme solution was applied on to a Sephadex G-75 column (2.6 cm x 100 cm), equilibrated with PBS at pH 7.2. The column was eluted with the

same buffer at a flow rate of 0.25 ml/min. The fraction with SOD activity was eluted with 55 ml PBS and dialyzed against 0.01M phosphate buffer pH 7.2 over night and concentrated with Amicon PM–30 (30,000-molecular weight cut off) and stored at -20ºC until used.

**SOD activity assay**

Superoxide dismutase activity was assayed after each purification step by measuring the inhibition of pyrogallol autoxidation rate.[13] The assay mixture contained 0.2mM pyrogallol in air equilibrated 50mM tris–cacodylic acid buffer, pH 8.2, and 1mM diethylene triamine penta acetic acid. The rate of autoxidation was obtained by monitoring the increase in absorbance at 420 nm in a Hitachi spectrophotometer, No U2000. SOD has the ability to inhibit the autoxidation and the extent of inhibition is taken as the measure of enzymic activity.

**Molecular Weight Determination**

The determination of the molecular weight by gel filtration was carried out on Sephadex G-200 column equilibrated with 0.05 M phosphate buffer pH 7.2, and calibrated with the following molecular weight standards: immunoglobulin G (160 000 D), human serum albumin (67 000 D), ß-lactoglobulin (35 000 D), cytochrome c (12 400 D), vitamin B12 (1355 D), cytidine (246 D).

**Protein Estimation**

Proteins were determined by the method of Bradford,[14] using bovine serum albumin as the standard.

**Activity staining for SOD**

Purified SOD was separated on a 10% non-denaturing polyacrylamide gel[15] for activity staining[16] Solution A was prepared by dissolving 10 mg nitroblue tetrazolium and 4 mg riboflavin in 10 ml glass distilled water. Solution B contained 600 µl TEMED in 10 ml glass distilled water. At the end of electrophoresis, gels were first incubated in Solution A for 20 min and then in Solution B for another 20 min to finally illuminate till the appearance of white bands against a blue background was observed.

This assay depends on the ability of SOD to inhibit the reduction of nitroblue tetrazolium to blue coloured formazan by $O_2^-$ generated by reoxidation of photochemically reduced riboflavin. Thus regions containing active SOD appear colourless against a blue background. The band intensity is roughly proportional to the amount of SOD protein.[16]

**SDS-Polyacrylamide gel electrophoresis**

SDS-PAGE of different fractions was done as described by Laemmli.[15]

## RESULTS AND DISCUSSION

**Purification of SOD**

After each purification step, the protein content and the enzyme activity were determined. According to our results, ammonium sulphate precipitation to 30% and 60% saturation eliminated 50% of the blast protein, without loss in SOD activity and the third precipitation step at 90% ammonium sulfate saturation resulted in a SOD preparation of higher specific activity.

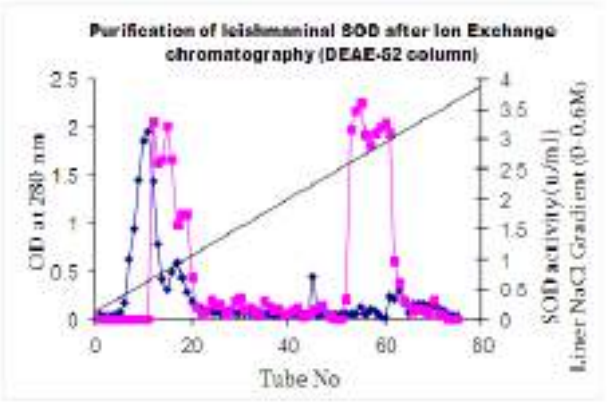


**Fig. 1: Ion-exchange chromatography on a DEAE-52 column; protein concentration (-▲-), superoxide dismutase activity (–●–), concentration of NaCl (—).**

After that, ion-exchange chromatography on DEAE-52 column with a concentration gradient of NaCl (0–0.6 M) was performed which resulted in elimination of 90% of the blast protein with a loss of SOD activity of about 57 %. Results of this chromatographic procedure are shown in Fig. 1.

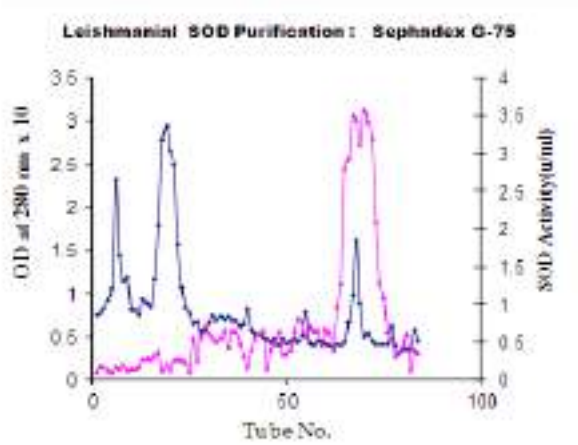


**Fig. 2: Gel chromatography on a Sephadex G-75 column; protein concentration (-▲-), superoxide dismutase activity (–●–).**

In Ion-exchange chromatography, the 28-44 eluted fraction detected at 280 nm presented an SOD activity and corresponds therefore to the SOD fractions. [Column Volume: 88 ml, Sample charged: 2 ml, Protein content of the sample: 0.47mg/ml, Active Fraction: Tube No. 65 to 72, Flow Rate: 1ml/min, Vol. of each tube: 2 ml].

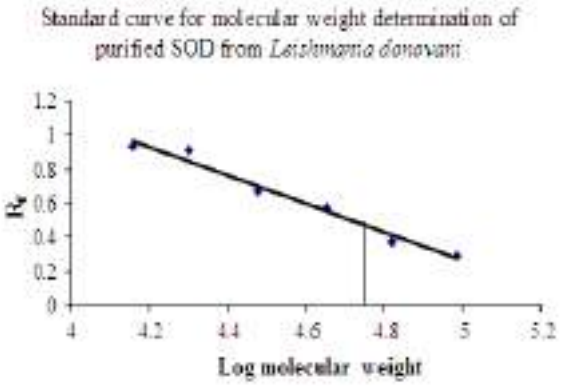


**Fig 3: Plot for determination of molecular weight of leishmanial SOD.**

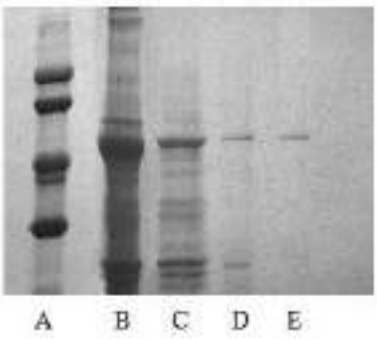


*Figure 4: SDS PAGE : A–Protein markers, B–Leishmania cell-free extract, C–Pellet after ammonium sulfate precipitation at 90%saturation, D–Fractions with SOD activity after ion-exchange chromatography, E-Fractions with SOD activity after gel chromatography*

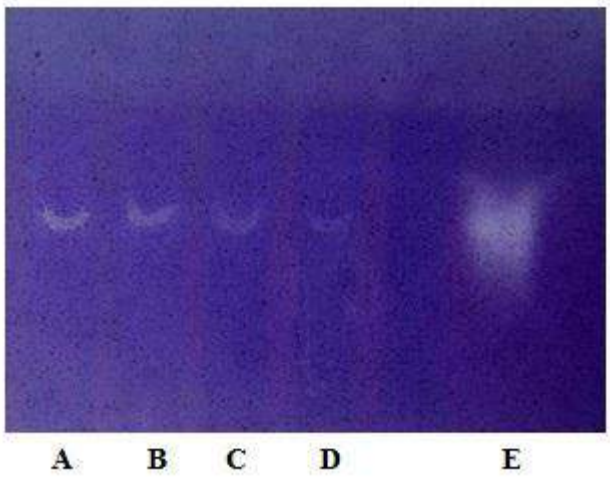


**Fig 5: Activity staining of leishmanial SOD after separating by native PAGE in a 10% gel. 60 micrograms of protein was loaded in each lane. A-Leishmania cell-free extract, B-Pellet after ammonium sulfate precipitation at 90%saturation, C-Fractions with SOD activity after ion-exchange chromatography, D-Fractions with SOD activity after gel chromatography, E-Pure Fe SOD.**

The SOD was then eluted from a Sephadex G-75 column. Figure 2 presents the results of the gel chromatography on Sephadex G-75 column. The pooled fraction had a volume of 20 ml and was concentrated to a 1.5 ml volume. The SOD fraction showed only one band of SDS-PAGE after gel chromatography. It can be concluded that the SOD was purified to homogeneity after three-step ammonium sulphate precipitation, ion exchange chromatography and gel chromatography. The final sample was applied on SDS-PAGE to determine its molecular weight (Fig 3). The molecular weight of SOD by SDS-PAGE was 37200 dalton. Figure 4 shows the electrophoretic pattern of SOD obtained during different steps of SOD purification. The total purification achieved by the procedure was 105 fold over the first soluble extract, with a yield of 15%. The specific activity of the purified enzyme was 9191 U/mg. Figure 5 shows the activity staining of leishmanial SOD after separating by native PAGE in a 10% gel. Activity staining of native polyacrylamide gels clearly showed the SOD activity obtained during different steps of SOD purification. SOD catalyzes dismutation of toxic superoxide radicals.[17] It is one of the key enzymes of oxygen defence system is known to be an essential factor in mediating normal cellular functions.[18] As a result, the enzyme has been found to be targeted for the treatment of several diseases.[19-21] SOD also plays a vital role during host–parasite interaction.

Its activity is elevated when Leishmania parasite infects host cells.[22] In a recent report, SOD has been demonstrated to be a key enzyme to play a vital role in the survival of intracellular parasites.[23] Importance of this enzyme in the host–parasite interaction was established by generating SOD-deficient *Leishmania donovani*. An earlier report suggested that the enzyme that was present in leishmanial glycosomes isolated by the classical sucrose gradient technique was of the Cu/Zn type.[16] In this present work, SOD was purified from *Leishmania donovani* promastigotes. The total purification achieved by the procedure was 105 fold over the first soluble extract, with a yield of 15%. The specific activity of the purified enzyme was 9191 U/mg. The molecular weight was estimated as 37200 dalton. SOD activity was observed in crude extracts of *Leishmania donovani* cells grown anaerobically. This finding indicates that these enzymes are being constitutively expressed during the growth of this organism in the absence of oxygen. Results elucidated the important roles of Fe-SOD in the cellular stress responses and anti-oxidative processes of the parasite *Leishmania donovani*. Future studies will be necessary to investigate functions of more antioxidant enzymes to gain a better understanding of the antioxidant mechanism in the Leishmania species under various stresses.

**ACKNOWLEDGEMENTS**

Authors are thankful to Dr. Md. Abdur Razzaque, Principal, Ananda Chandra College, Jalpaiguri, for his keen interest in this study. University Grants

Commission, New Delhi is acknowledged for offering Senior Research Fellowship to R. Jyoti through Rajiv Gandhi National Fellowship (RGNF) Scheme. Financial support from Department of Science and Technology, West Bengal, is also duly acknowledged.

**REFERENCES**

1. Ameziane-El-Hassani, R. and Dupuy, C. Detection of reactive oxygen species in cells undergoing oncogene-induced senescence. *Methods Mol Biol.*, 2017; 1534: 139-45.
2. Grace, SC. Phylogenetic distribution of superoxide dismutase supports an endosymbiotic origin for chloroplasts and mitochondria. *Life Sci.*, 1990; 47: 1875-86.
3. Phan, IQ., Davies, DR., Moretti, NS., Shanmugam, D., Cestari, I., Anupama, A., Fairman, JW., Edwards, TE., Stuart, K., Schenkman, S. and Myler, PJ. Iron superoxide dismutases in eukaryotic pathogens: new insights from apicomplexa and Trypanosoma structures. *Acta Crystallogr F Struct Biol Commun.*, 2015; 71: 615-21.
4. Fernandez-Ocana, A., Chaki, M., Luque, F., Gómez-Rodríguez, MV, Carreras, A., Valderrama, R., Begara-Morales, JC., Hernández, LE., Corpas, FJ. and Barroso, JB. Functional analysis of superoxide dismutases (SODs) in sunflower under biotic and abiotic stress conditions. Identification of two new genes of mitochondrial Mn-SOD. *J Plant Physiol.* 2011; 168: 1303-8.
5. Youn, HD., Kim, EJ., Roe, JH., Hah, YC. And Kang, SO. A novel nickel-containing superoxide dismutase from Streptomyces spp. *Biochem J.*, 1996; 318: 889-96.
6. Heyworth, PG., Cross, AR. and Curnutte, JT. Chronic granulomatous disease. *Curr Opin Immunol*, 2003; 15: 578-84.
7. Umasuthan, N., Bathige, SD., Revathy, KS, Lee, Y., Whang, I., Choi, CY, Park, HC. and Lee, J. A manganese superoxide dismutase (Mn-SOD) from *Ruditapes philippinarum*: comparative structural and expressional-analysis with copper/zinc superoxide dismutase (Cu/Zn-SOD) and biochemical analysis of its antioxidant activities. *Fish Shellfish Immunol*, 2012; 33: 753-65.
8. Lynch, M. and Kuramitsu, H. Expression and role of superoxide dismutases (SOD) in pathogenic bacteria. Microbes Infect, 2000; 2: 1245-55.
9. Battistoni, A. Role of prokaryotic Cu-Zn superoxide dismutase in pathogenesis. *Biochem. Soc. Trans*, 2003; 31: 1326-9.
10. Gupta, S., Raychaudhury, B. and Datta, SC. Host peroxisomal properties are not restored to normal after treatment of visceral leishmaniasis with sodium antimony gluconate. *Exp Parasitol*, 2009; 123: 140-5.
11. Jaffe, CL., Grimaldi, G. and McMahon-Pratt, D. In: Morel, CM. (Ed.), Genes and Antigens of Parasites: A Laboratory Manual, Fundaao Oswaldo Cruz, Rio de Janeiro, 1984; 47.
12. Ray, JC. Cultivation of various Leishmania parasites on solid medium, *Ind. J. Med. Res*, 1932; 20: 355–7.
13. Raychaudhury, B. *In vitro* antileishmanial activity of *Nyctanthes arbor-tristis* – a medicinal tree. *International Journal of Applied Biology and Pharmaceutical Technology*, 2013; 4: 146-51.
14. Bradford, MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*, 1976; 72: 248-54.
15. Laemmli, UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 1970; 227: 680-5.
16. Raychaudhury, B., Gupta, S., Banerjee, S., Das, B. and Datta, SC. Isolation of *Leishmania* glycosomes by a rapid method. *Analytical Biochemistry*, 2004; 332: 404–8.
17. Raychaudhury, B., Banerjee, S., Gupta. S., Singh, RV. And Datta, SC. Antiparasitic activity of a triphenyl tin complex against *Leishmania donovani*. *Acta Tropica*, 2005; 95: 1–8.
18. Fattman, CL., Schaefer, LM. and Oury, TD. Extracellular superoxide dismutase in biology and medicine. *Free Radic. Biol. Med.*, 2003; 35: 236–56.
19. Yassien, N.A., Khalifa, EA. and el-Nouby, KA. Role of antioxidant preparation (oxi-guard) on *Cryptosporidium parvum* infection in albino mice. *J. Egypt. Soc. Parasitol.*, 2001; 31: 95–106.
20. Briedbach, T., Scory, SR., Krauth-Siegel, L. and Steverding, D. Growth inhibition of bloodstream forms of *Trypanosoma brucei* by the chelator deferoxamine. *Int. J. Parasitol.*, 2002; 32: 473–9.
21. Mohan, S., Pradeep, CG, Abhilash, KR., Jayakumar, K. and Raj, KR. The adult-specific ubiquinone Q (8) functions as an antioxidant in the filarial parasite, *Setaria digitata*. *Biochem. Biophys. Res. Commun*, 2000; 288: 949–53.
22. Dey, R., Mitra, S. and Datta, SC. Cyclic AMP mediates change in superoxide dismutase activity to monitor host–parasite interaction in *Leishmania donovani*. *J. Parasitol*, 1995; 81: 683–6.
23. Ghosh, S., Goswami, S. and Adhya, S., Role of superoxide dismutase in survival of *Leishmania* within the macrophage. *Biochem. J*, 2003; 369: 447–52.