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Pharmacological assessment of the heartwood of *Acacia raddiana* Willd for antifungal potential

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ABSTRACT

As COVID-19 suppresses the immune system and those who have recovered from COVID-19 are at risk of developing mucormycosis or black fungus so there is a need to develop new antifungal strategies by the use of medicinal plants. Medicinal plants have always been a subject of interest for drug discovery, ethnobotany, traditional and indigenous medicines. One of the successful strategies for the investigation of new bioactive compounds from medicinal plants includes the screening of plant extract, fractions followed by the purification of the constituents and screening for biological activity. In the present study, the heartwood of desert plant *Acacia raddiana* Willd was screened for antifungal activity by Agar-well diffusion method against *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Penicillium chrysogenum* and *Trichophyton rubrum*. The result obtained shows that heartwood extract and their fractions serve as an effective agent against selected fungi and efficiency is dependent upon the nature of fraction and vary with respect to specific fungi. The extract and fractions shows a wide antifungal potential against *C. albicans*. The findings suggest that the medicinal plant under investigation might be a reasonable solution for fungal infections especially against *C. albicans*.

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1. Introduction

Plants have been utilized as a source of pharmaceuticals from ancient times, and as of today, around 70,000 species have been assessed for their potential value as medicines. Plant-based remedies play an important role in the healing process in Ayurveda. In Ayurveda, any plant, animal or mineral substance can be used for therapeutic benefits. Plants have an important function in the development of therapeutic medicines, as per findings in present medicinal fields of science. The first book on screening natural compounds for drug development was Principals of Medicinal Chemistry [1]. Dev (1989) described important genera such as *Datura*, *Duboisia* spp., *Solanumdulcamara*, *Atropa belladonna* and *N. tobaccum* being utilized as a source of modern drugs [2]. Several local plant extracts have been marked for their biological activities. Aneja & Joshi evaluated antimicrobial properties of fruit extracts

of Terminaliachebula, commonly known as Harad that has multiple health benefits [3]. Park et al. studied the pharmacological actions of Cactus [4]. Kotkar et al. investigated the antimicrobial and pesticide activity of partially purified flavonoids of *Annona squamosa*, commonly known as custard apple or Seetafal [5]. Tahir et al. investigated selected Sudanese medicinal plants for their anti-plasmodial activity [6]. Darshan & Doreswamy researched on traditional medicine for the anti-inflammatory plant drug development [7]. In the present study desert, plant selected for antifungal bioassay belongs to the genus *Acacia*, which is commonly known as the Wattles or Acacias. Several species of *Acacia* are also known for antimicrobial, antifungal, anticancer, and antioxidant activities. Arias et al. investigated the antibacterial activity of seven ethanol extracts and three aqueous extracts from various parts (leaves, stems, and flowers) of *A. aroma* against strains of antibiotic multi-resistant bacteria [8]. The result showed that all ethanolic extracts had activity against gram-positive bacteria but against Gram-negative bacteria, only leaf and flower fluid extracts showed activity. Cavazos et al. determined the antibacterial and antioxidant activity of *Acacia berlandieri* and *Acacia*

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rigidula leaves [9]. Madjid et al. investigated the chemical diversity and pharmacological properties of the genus *Acacia* in 2020 [10]. Uzunugbe et al. identified phenolic and flavonoid constituents and antioxidant activities of crude extracts from *Acacia Senegal* leaf extracts [11]. Borges et al. phytochemically investigated the *Acacia dealbata* and identified its antimicrobial and antioxidant potential [12]. Root and stem extract of *Acacia rigidula* had been used as a reducing and capping agent to produce silver nano-particles that eradicate pathogenic resistant bacteria in vivo

[13]. *Acacia raddiana* is a medicinal desert plant, that has been investigated phyto-chemically mainly for poly-phenols [14]. Plants containing phenolic compounds are a possible source of natural antioxidants that stabilize free radicals by hydrogenation or complexing with oxidizing species [15]. Various extracts of this plant showed muscle relaxing activity [16] while its seed extract exhibited anti-hyperglycemic activity [17]. Leaves of this plant afforded ellagitannin, galloylglucoside, flavonol glycosides [18]. Its stem bark yielded n-hexacosanol, betulin, α -amyrin, β -sitosterol, β -

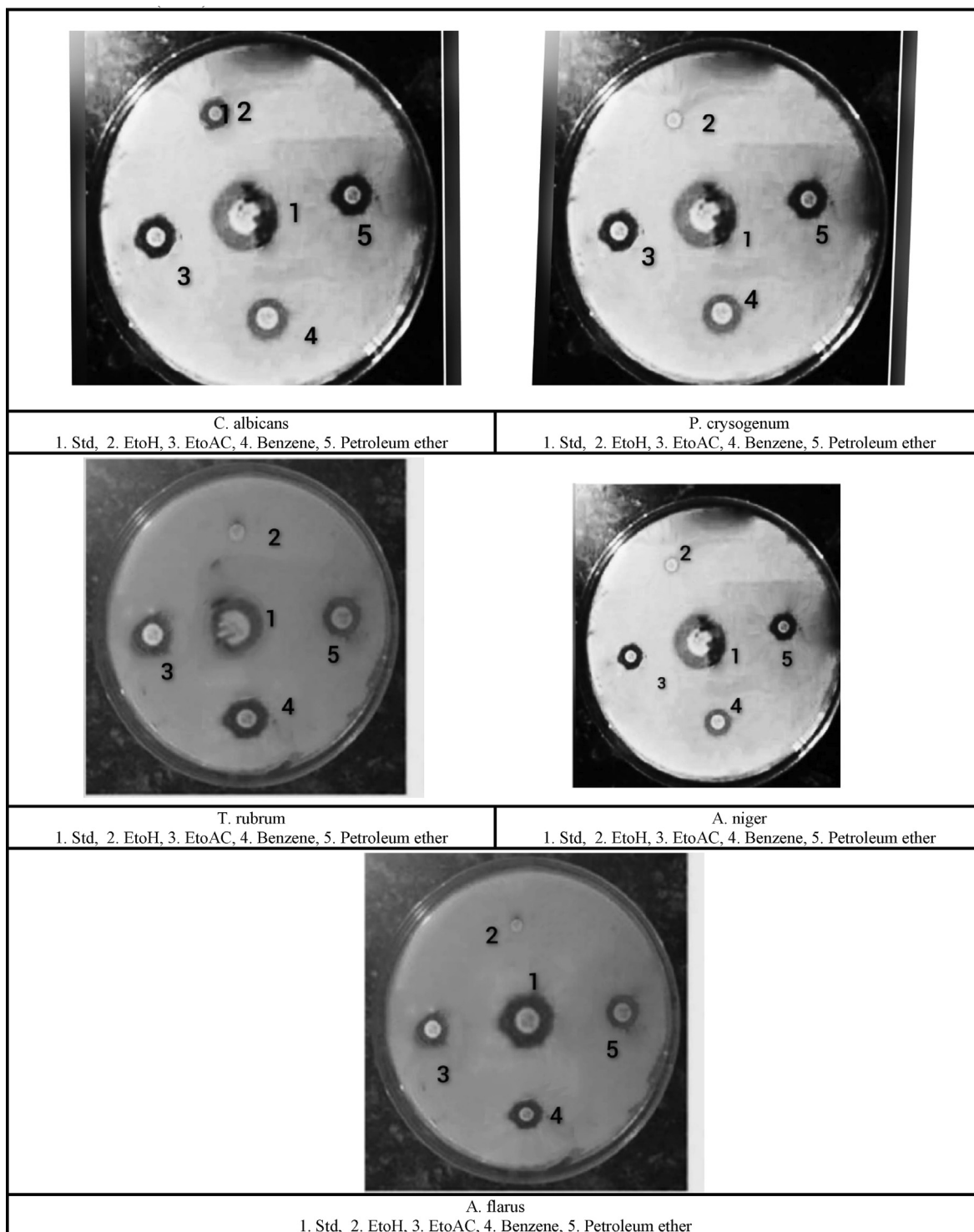


Fig.1. Inhibition zone obtained by agar well diffusion method.

amyrinand n-octacosanol, 3-acetyl- β -sitosterol, γ -sitosterol, betulin, friedelin have been isolated from its heartwood [19]. We obtained a dark brownish semi-solid mass (185 g) by extraction with ethanol. Which was divides in two portions, first portion (120 g) used for phytochemical investigation and second portion (65 g) for antifungal screening. First portion column chromatographed over silica gel (60–120 mesh). Elution was carried out with solvents of increasing polarity viz., petroleum ether, benzene, ethyl acetate and ethanol. Several fractions were collected and crystallized. These fractions gave nine known compounds (Octacosanol, Monacosanol, β -Sitosterol octacosanoate, β -Sitosterol acetate, α -Amyrin, β -sitosterol, Betulin Friedelin, D-Pinitol) along with one unknown compound. In present study, we represent the antifungal screening of the heartwood extract and fractions. Fungal infection is a serious problem for our health system. Now, there is a public criticism of synthesized chemicals that are used as safe and strong antifungal agent. Fungi are found to occur everywhere in the environment, which are unavoidable and cause infection in plants as well as animals. Although over 600 species of fungi have been found to cause disease, only roughly 20 fungi are responsible for > 90 percent of human fungal infections. *Candida* and *Aspergillus* species are the most frequent fungal pathogens that cause invasive, life-threatening illnesses. Hence, all screening assays include these fungi. In spite of a large number of antifungal drugs in the market, there remains a need of drugs, which are more effective and exhibit broad spectrums efficacy. In the present, the antifungal activities of the heartwood of *Acacia raddiana* Willd had been evaluated for its ethanolic extract (EtOH). Test fungi *Aspergillus flavus* (*A. flavus*), *Aspergillus niger* (*A. niger*), *Candida albicans* (*C. albicans*), *Penicillium chrysogenum* (*P. chrysogenum*), and *Trichophyton rubrum* (*T. rubrum*) were screened by agar-well diffusion method [20] using ketoconazole as standard.

2. Materials and methods

2.1. Plant collection

The plant used in this study is *Acacia raddiana* Willd of the family Fabaceae, subfamily Mimosaceae. Further, it is the most significant species in hot and arid environments and it is widely

employed in all climate regions where it is environmentally suitable. The plants samples were detect taxonomically and identified. The plants were then dries at room temperature (31 °C) and the powdered materials were ground using an electric mixer. Air-dried and coarsely powdered heartwood (8.2 kg) shaving was extract with ethanol and gave a dark brownish semi-solid mass (185 g), which was divided in two portion, first portion (120 g) used for phytochemical investigation and second portion (65 g) for antifungal screening.

2.2. Source of test organisms

Pure cultures of test fungi namely *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Penicillium chrysogenum* and *Trichophyton rubrum* obtained from SMS Lab. Jaipur (Rajasthan) were cultured on Sabouraud Dextrose Broth (SDB) at 37 °C for 48 h.[21]

2.3. Antifungal assay -Agar well diffusion method

Stock cultures were maintained at 4 °C on slops of nutrient agar. Active cultures for experiments were prepared by transferring an inoculating loop of cultures from the stock cultures to test tubes of SD Broth, which were incubates without agitation for 24 h at 25 °C. The holes plate diffusion method is another name for the agar well diffusion method [22]. The culture media for this experiment was nutrient agar. The molten nutritional agar was pour into pre-sterilized Petri dishes and left to settle. The test microorganism was suspend in SD broth and homogeneously infused onto these agar media. The samples were lefts to solidify. With the use of a sterile stainless steel borer, holes/wells of 6 mm diameter were bored into agar following solidification. For every plate, 5 wells were made 4 holes were filled with 0.2 ml of plant extract and also the 5th hole with 0.2 ml of standard ketoconazole solution. Sample plates were incubates for 36 h at 37°Celsius. After this period, the diameter of the inhibitory zone produced (Fig. 1). The distance between each hole was measure and the values were record. The antifungal activity was given as a ratio by the inhibition zone generated by the plant extract and the inhibition caused by the control drug ketoconazole was employed (Fig. 2). The activity index was calculate by using following equation.

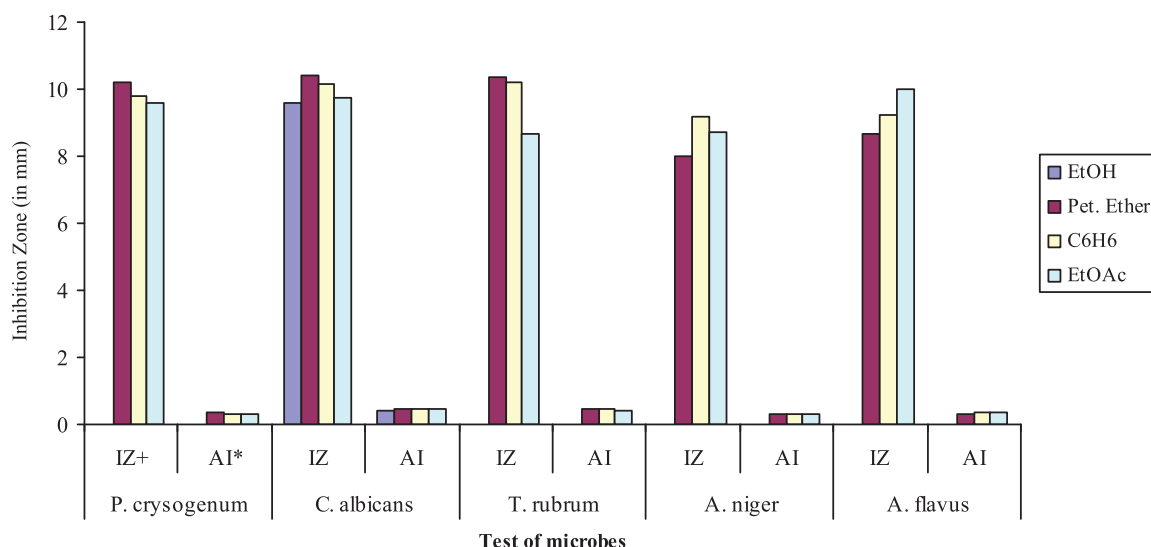


Fig. 2. Antifungal activity of the heartwood of *Acacia raddiana* Willd.

Table 1
Antifungal activity of the heartwood of *Acacia raddiana* Willd.

Plant Species	Type of extract/ fractions	Dose (mg/ disc)	Test Microbes									
			<i>P. crysogenum</i>		<i>C. albicans</i>		<i>T. rubrum</i>		<i>A. niger</i>		<i>A. flavus</i>	
			IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
<i>Acacia raddiana</i> Willd(Heartwood)	EtOH	4	–	–	9.60	0.43	–	–	–	–	–	–
	Petroleum Ether	4	10.20	0.35	10.42	0.48	10.34	0.47	8.00	0.30	8.68	0.32
	Benzene	4	9.80	0.33	10.13	0.45	10.21	0.46	9.20	0.33	9.25	0.34
	EtOAc	4	9.60	0.32	9.76	0.44	8.68	0.40	8.74	0.32	10.00	0.36

AI = Inhibition zone of sample/Inhibition zone of standard

IZ = Inhibition zone (in mm), with the diameter of disc (6 mm);

Standard: Ketoconazole;

(-) = No activity.

AI = Activity index = Inhibition zone of sample/Inhibition zone of standard;

3. Result and discussion

In the present study five-test fungi namely *P. crysogenum*, *C. albicans*, *T. rubrum*, *A. niger* and *A. flavus*, were used to screen the possible antifungal activity of plant extract. The results of antifungal activities of the selected plant extract and fractions (ethanol, benzene, petroleum ether, ethyl acetate) have been presenting in Table 1 and Fig. 1. The activities were visible as an inhibition zone produced around every well, the diameter of which assesses the level of inhibition. The size of the inhibitory zone in each extract against each fungus is significantly different. The result showed that the ethanolic extract of heartwood of *Acacia raddiana* Willd. Exhibited not much effect against the selected test fungi except moderate activity against *C. albicans* (IZ = 9.60, AI = 0.43). Petroleum ether fraction show effectiveness against all test fungi but show wide potential against *C. albicans* (IZ = 10.42, AI = 0.48) and *T. rubrum* (IZ = 10.34, AI = 0.47). The ethanolic extract of heartwood of *Acacia raddiana* Willd. did not exhibit much effect against any of the selected test fungi except moderate activity against *C. albicans* (IZ = 9.60, AI = 0.43). Petroleum ether fraction show effectiveness against all test fungi but show wide potential against *C. albicans* (IZ = 10.42, AI = 0.48) and *T. rubrum* (IZ = 10.34, AI = 0.47). Benzene fractions show effectiveness against all test fungi but show wide potential against *C. albicans* (IZ = 10.13, AI = 0.45) and *T. rubrum* (IZ = 10.21, AI = 0.46).

4. Conclusion

Currently, “Black Fungus” is a new source of concern after the Corona virus pandemic. In the majority of its cases, immediate surgical debridement to remove diseased necrotic tissue is recommended, followed by antifungal medication and in the search for naturally antifungal drug production researchers has the focus on medicinal plants extracts and its antifungal bioassay Therefore, screening of medicinal plants and herbs are required for discovering new antimicrobial agents with wide potential. In this study, antifungal screening of the heartwood of *Acacia raddiana* Willd. was done by the agar well diffusion method. The result showed that the ethanolic extract of the heartwood of *Acacia raddiana* Willd. has effective antifungal activity only against *C. albicans*. Other than this, all other fractions showed wide potential against *C. albicans* and also against *T. rubrum*. Therefore, from this study it is concluded that an important antibiotic against *C. albicans* might be yield from the heartwood of *Acacia raddiana* Willd. Further investigations are necessary to evaluate the potential against other infectious diseases, especially for antiviral diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] W.O. Foye, Screening of natural products for drug discovery", Principals of Medicinal Chemistry, (1981) 697.
- [2] S. Dev, Higher Plants as a source of drugs, in: Plant and Society, Macmillan Publisher Ltd, London, 1989, pp. 267–292.
- [3] K.R. Aneja, R. Joshi, Evaluation of antimicrobial properties of fruit extracts of Terminalia chebula against dental caries pathogens, Jundishapur J. Microbiol. 2 (3) (2009) 105.
- [4] E.H. Park, J.H. Kahng, E.A. Paek, Studies on the pharmacological actions of cactus: identification of its anti-inflammatory effect, Arch. Pharmacol Res. 21 (1) (1998) 30–34.
- [5] H.M. Kotkar, P.S. Mendki, S.V.G.S. Sadan, S.R. Jha, R. Shipra, S.M. Upasani, V.L. Maheshwari, Antimicrobial and pesticidal activity of partially purified flavonoids of Annonasquamosa, Pest Manag. Sci. 58 (2002) 33–37.
- [6] A. El Tahir, G.M.H. Satti, S.A. Khalid, Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on Maytenus senegalensis (Lam.) Exell., J. Ethnopharmacol. 64 (3) (1999) 227–233.
- [7] S. Darshan, R. Doreswamy, Patented ant-inflammatory plant drug development from traditional medicine, Phytotherapy Res. 18 (5) (2004) 343–357.
- [8] M.E. Arias, J.D. Gomez, N.M. Cudmani, M.A. Vattuone, M.I. Isha, Antibacterial activity of ethanolic and aqueous extracts of Acacia aroma Gill, ex Hook et Arn.; Life sciences 75 (2) (2004) 191–202.
- [9] P. Cavazos, D. Gonzalez, J. Lanorio, R. Ynalvez, Secondary metabolites, antibacterial and antioxidant properties of the leaf extracts of Acacia rigidula benth. and Acacia berlandieri benth., SN Appl. Sci. 3 (5) (2021).
- [10] A. Madjid O., A. Sanni, L. Lagnika, Chemical Diversity and Pharmacological Properties of Genus Acacia, Asian J. of Applied Sciences 13 (2) (2020) 40–59.
- [11] E.O. Uzunuigbo, F.O. Osunsanmi, P. Masamba, R.O. Mosa, A.R. Opoku, A.P. Kappo, Phytochemical constituents and Antioxidant Activities of Crude Extracts from Acacia Senegal Leaf Extracts, Pharmacognosy Journal 11 (2019) 1409–1414.
- [12] A. Borges, H. José, V. Homem, M. Simões, Comparison of techniques and solvents on the antimicrobial and antioxidant potential of extracts from Acacia dealbata and oleaeuropea, Antibiotics (2020), <https://doi.org/10.3390/antibiotics9020048>.
- [13] C.E. Escárcega-González, J.A. Garza-Cervantes, A. Vazquez-Rodríguez, L.Z. Montelongo-Peralta, M.T. Treviño-Gonzalez, E.D.B. Castro, E.M. Saucedo-Salazar, R.C. Morales, D.R. Soto, F.T. González, J.C. Rosales, In vivo antimicrobial activity of silver nanoparticles produced via a green chemistry synthesis using Acacia rigidula as a reducing and capping agent, Int. J. Nanomed. 13 (2018) 2349.
- [14] L. Boulus, Michigan, a Medicinal Plants of North Africa, Reference Publications Inc, Algonac, 1983, p. 286.
- [15] T. Afsar, S. Razzak, M. Shabbir, M.R. Khan, Antioxidant activity of polyphenolic compounds isolated from ethyl-acetate fraction of Acacia hydasppica R, Parker. Chemistry Central Journal 12 (1) (2018) 1–13.
- [16] M. Hagos, G. Samuelsson, L. Kenne, B.M. Modawi, Isolation of smooth muscle relaxing 1,3-diaryl- propan-2-ol derivatives from Acacia tortilis, Planta Med. 53 (1) (1987) 27–31.
- [17] N.K. Agarwal, U. Gupta, Evaluation of hypoglycemic and antihyperglycemic effects of Acacia tortilis seed extract in normal and diabetic rats, International Journal of Pharm Tech Research 5 (2) (2013) 330.

- [18] A.M.D. El-Mousallomy, H.H. Barakat, A.M.A. Souleman, S. Awadallah, Polyphenols of *Acacia raddiana*, *Phytochemistry* 30 (11) (1991) 3767–3768.
- [19] H.M. Muhaisen, A Review on Chemical Constituents of *Acacia Tortilis* (Leguminosae), *IOSR Journal of Pharmacy* 11 (3) (2021) 10–21.
- [20] L. Boyanova, G. Gergova, R. Nikolov, S. Derejian, E. Lazarova, N. Katsarov, I. Mitov, Z. Krastev, Activity of Bulgarian propolis against 94 *Helicobacter pylori* strains in vitro by agar-well diffusion, agar dilution and disc diffusion methods, *J. Med. Microbiol.* 54 (5) (2005) 481–483.
- [21] C. Perez, I. Albert, K. DeFay, N. Zachariades, L. Gooding, M. Kriegler, A nonsecretable cell surface mutant of tumor necrosis factor (TNF) kills by cell-to-cell contact, *Cell* 63 (2) (1990) 251–258.
- [22] A. Brantner, K. Pfeiffer, E. Grein, Antibacterial Assays of the Pharmacopoeias: diffusion Tests of Natural Substances and their Evaluation, *Planta Med* 59 (S 1) (1993) A675.