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ANTIMICROBIAL ACTIVITY OF *Hibiscus sabdariffa* AND *Sesbania grandiflora* EXTRACTS AGAINST SOME G–ve AND G+ve STRAINS

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Abstract. Medicinal plants such as *Hibiscus sabdariffa* and *Sesbania grandiflora* are widely used to treat different diseases caused by different microbes in several countries. The antimicrobial activities of *Hibiscus* and *Sesbania* extracts were examined in present work. The antimicrobial activity of the plant extracts were investigated against *Escherichea coli*, *Staphyloccoccus* aureus and *Pseudomonas aeruginosa*. This study exhibited strong antibacterial activity of the aqueous extract of *H. sabdariffa* in comparison to *S. grandiflora* stem and root ethanolic extracts. Results obtained from this study indicate that both extracts exhibited concentration dependent effect. *Hibiscus* extract showed the highest inhibition activity against all bacterial strains at concentration 500 mg/mL in comparison to *Sesbania* extract.
Keyword*: Antimicrobial activity, Hibiscus sabdariffa, Inhibition zone, Sesbania grandiflora*.

Introduction

Many naturally occurring compounds found in plants have been revealed to own biological activities including anti–allergic [PANICHAYUPAKARANANT *et al.,* 2010, ANANTAWORASAKUL *et al.,* 2011], anti–cholinesterase [ELDEEN *et al.,* 2005, ANANTAWORASAKUL *et al.,* 2011], antibacterial [OZCELIK *et al.,* 2005, ELDEEN *et al.,* 2005, ANANTAWORASAKUL *et al.,* 2011], antifungal [OZCELIK *et al.,* 2005, KUETE *et al.,* 2010, MATIVANDLELA *et al.,* 2006, ANANTAWORASAKUL *et al.,* 2011; BAGIU et al., 2012], antiviral [OZCELIK *et al.,* 2005, GLATTHAAR–SAALMULLER *et al.,* 2001, ANANTAWORASAKUL *et al.,* 2011], antioxidant [AHMAD *et al.,* 2005, OKONOGI *et al.,* 2007, ANANTAWORASAKUL *et al.,* 2011], anti–inflammatory [PANICHAYUPAKARANANT *et al.,* 2010, ELDEEN *et al.,* 2005, ANANTAWORASAKUL *et al.,* 2011, BOONKAEW CAMPER, 2005], antitumor [LAMPRONTI *et al.,* 2006, HAQUE *et al.,* 2000, ANANTAWORASAKUL *et al.,* 2011], cytotoxic effects [BOONKAEW CAMPER, 2005, KAMATOU *et al.,* 2008, ANANTAWORASAKUL *et al.,* 2011], antityrosinase [LEE *et al.,* 2011, ANANTAWORASAKUL *et al.,* 2011] and anti–plasmodial [KAMATOU *et al.,* 2008, ANANTAWORASAKUL *et al.,* 2011].

Medicinal plants are widely used in several countries such as Thailand and other Asian countries like China, Japan, and India either directly or indirectly.

The world health organization (WHO) has accepted so far, an inventory of more than 20,000 species of medicinal plants.

They or their products are used in controlling various diseases and disorder symptoms as cough, fever, bronchitis, itching, pneumonia, ulcers, and diarrhea [ANANTAWORASAKUL *et al.,* 2011].

Hibiscus sabdariffa is reported as one of the most important medicinal plants that have been widely used.

It is an annual, tropical or subtropical shrub grown in many countries including India, Thailand, Sudan, and Mexico.

The calyces of *H. sabdariffa* are used in the preparation of a beverage whereas many species of *Hibiscus* are used as ornamentals [HIGGINBOTHAM *et al.,* 2014].

Hibiscus extracts have been demonstrated to have a wide range of antimicrobial activity against many bacterial strains, for instance.

Methanol extractions of the calyces have established to possess antimicrobial activity against each of *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Serratia marcescens*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas* sp. at different concentrations [HIGGINBOTHAM *et al.,* 2014, PRESCOTT *et al.,* 2002; RASHED and BUTNARIU, 2014a].

Likewise *Sesbania* herb, which is one of the Leguminosae family, consists

of about 60 species that are widely distributed throughout tropical and subtropical regions. Most of its species are annual herbs or shrubs, nevertheless a few are small trees [BURGESS *et al.*, 1990, ANANTAWORASAKUL *et al.*, 2011]. Furthermore, many species of *Sesbania* are playing important role in soil improvement as green manners or agroforestry trees [PALM *et al.*, 2001, ANANTAWORASAKUL *et al.*, 2011].

In Thailand *S. grandiflora* has been widely used both for food and medicine.

Both, leaves and flowers of *S. grandiflora* are utilized as food whereas its stem has been long used as a traditional medicine for treatment of ulcers in the oral cavity.

It has been stated that all parts of *S. grandiflora* such as roots, bark, gum, leaves, flowers and fruits are used for medicine in Southeastern Asia and India.

The juice of the leaves has been considered as anthelmintic and tonic and has been used to treat worms, fever and itchiness [ANANTAWORASAKUL *et al.*, 2011].

S. grandiflora powdered roots in an appropriate amount of water have been established to decrease the rheumatic swelling when applied externally with moderate rubbing to the lesion [KASTURE *et al.*, 2002, ANANTAWORASAKUL *et al.*, 2011].

In addition, the bark has used as astringent and utilized to treat cough, vomiting, bronchitis, ulcers, diarrhea, dysentery and dental caries [PRESCOTT *et al.*, 2002, KACHROO VIPIN *et al.*, 2011].

Hence *Hibiscus* and *Sesbania* extracts were used to treat different diseases caused by different microbes including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

These bacterial strains cause several diseases, for example, Some of *E. coli* strains can cause disease in healthy individuals such as enteric/diarrheal disease, urinary tract infections (UTIs) and sepsis/meningitis [NATARO KAPER, 1998, KAPER *et al.*, 2004].

The G–ve *E. coli* naturally colonizes the gastrointestinal tract of mammals, including humans in usually a commensalism relationship [KAPER *et al.*, 2004, ELENA *et al.*, 2005; BUTNARIU *et al.*, 2005; SAMFIRA *et al.*, 2013].

Pseudomonas aeruginosa, another G–ve bacterium, is an opportunistic pathogen that normally dwells the soil and surfaces in aqueous environments.

Its antibiotic resistance ability facilitates its survival in a wide range of other natural and artificial settings [GELLATLY HANCOCK, 2013]. Moreover, it also owns a remarkable aptitude to develop or acquire new antibiotics resistance mechanisms [MESAROS *et al.*, 2007]. *P. aeruginosa* infections are often nosocomial, and approximately all are accompanied by compromised host defenses such as in severe burns and cystic fibrosis [LYCZAK *et al.*, 2000, GELLATLY HANCOCK, 2013]. *P. aeruginosa* infections demonstrate high morbidity and mortality [GELLATLY HANCOCK, 2013].

Furthermore, Staphylococci are Gram–positive bacteria, many of which preferentially colonize the human body [KLOOS BANNERMAN, 1994, HARRIS *et al.*, 2002].

Generally, Staphylococci are able to tolerate high salt concentrations [WILKINSON, 1997] and exhibit heat resistance [ALBERT BALOWS *et al.*, 1991].

Pathogenic staphylococci are commonly having the ability to produce coagulase, and consequently cause blood clot [KLOOS MUSSELWHITE, 1975, HARRIS *et al.*, 2002].

S. aureus is found naturally on the skin and in the nasopharynx of the human body. As a result of the rise in antibiotic resistance, *S. aureus* is of increasing importance [LOWY, 2000].

This bacterium can cause local infections of the skin, nose, urethra, vagina and gastrointestinal tract [SHULMAN NAHMIAS, 1972, HARRIS *et al.*, 2002].

The aim of the present this study is to evaluate the antimicrobial activity of two selected plants; *Hibiscus sabdariffa* and *Sesbania grandiflora.* The antimicrobial activity of the plant extracts were estimated against *Escherichea coli*, *Staphyloccoccus aureus* and *Pseudomonas aeruginosa,* respectively.

Material and methods

Plant material

Hibiscus sabdariffa flowers were collected from local markets in Baghdad, Iraq. While, *Sesbania grandiflora* was

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gathered from gardens of University of Bagdad, Iraq.

Method

Preparation of Raw Material

Hibiscus sabdariffa flowers as well as stems and roots of *Sesbania grandiflora* were washed well with tap water then dried in shade.

The dried materials were eventually grounded to form a fine powder. Powdered plant material of *Hibiscus* was extracted with water as a solvent of extraction by maceration method while powdered stems and roots of *Sesbania* were subjected to ethanol 95 % for extraction by Soxholet apparatus.

Both crude extracts were concentrated by incubator with 40°C separately. Then the dried materials were weighted and dissolved in sterile distilled water. Two concentrations (250 and 500 mg/mL) for each extract were prepared.

Bacterial cultures preparation

In the meantime, growth cultures of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were prepared and incubated overnight at 37°C. Each bacterial culture was then suspended in 0.9% NaCl, and diluted to a McFarland standard No. 0.5 [NCCLS, 1992, ANANTAWORASAKUL *et al.*, 2011].

This bacterial suspension was used later to test the antibacterial activity.

Determination of antimicrobial activity

Disc diffusion assay [BAUER *et al.*, 1966, ANWAR IBRAHIM *et al.*, 2014; BUTNARIU *et al.*, 2014] was used in order to investigate the antimicrobial activity of the tested extracts. 0.1 mL of the bacterial suspensions was spread uniformly on nutrient agar plates.

Filter paper discs in 5.5 mm diameter were prepared and sterilized.

First group of these discs were loaded, by sterile micropipette, with 10 µL of 50 % ethanol [ANANTAWORASAKUL *et al.*, 2011].

Ten microliters of 30µg/mL streptomycin were loaded to a second group of discs as the inhibitory capability of streptomycin for both G–ve and G+ve was established [TODAR, 2004].

Hibiscus and *Sesbania* extracts were added to third and fourth group of sterile discs, respectively, with concentrations of (250 and 500 mg/mL) for each extract.

The loaded discs were allowed to dry, placed on the plates and subsequently incubated overnight at 37°C. The diameter of inhibition zone was measured in order to evaluate the antimicrobial activity.

The experiment was carried out in duplicate and the mean of diameter of the inhibition zone was calculated.

Results and discussion

Each extract was tested for its antibacterial activity against *E. coli* and *P. aeruginosa* as representations of Gram negative, while *S. aureus* represented Gram positive bacteria.

Table 1.

Plants Extracts at Different Concentrations and The corresponding Inhibition Zone of Some Bacterial Strains

Plant extract	Concentration mg/mL	Diameter of Inhibition zone (mm)[a]		
		Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Hibiscus sabdariffa flowers	250	13.55	11.61	8.45
	500	17.47	15.34	9.52
	Antibiotic[b]	21.71	29.51	26.67
Sesbania grandiflora stems	250	7.45	6.67	7.83
	500	9.46	8.91	9.81
	Antibiotic	24.97	31.8	27.95
Sesbania grandiflora roots	250	N.D[c]	8.51	N.D
	500	N.D	10.72	6.96
	Antibiotic	N.D	31.71	28.69

[a] data are represented as mean of 2 replications; [b] the used antibiotic is Streptomycin (30µg/mL); [c] N.D represents none determined inhibition zone

Streptomycin was used as a positive control. On the other hand, 50 % ethanol was considered as a negative control.

Results shown in Table 1 indicate that both extracts exhibited concentration dependent effect. *Hibiscus* extract showed the highest inhibition activity against all bacterial strains at concentration 500 mg/mL in comparison to *Sesbania* extracts with a diameter of inhibition zone of 17.47, 9.52 and 15.34 mm for *E. coli*, *P. aeruginosa* and *S. aureus*, respectively.

In general, the *H. sabdariffa* aqueous extract has shown more biological activity than the corresponding concentration of *S. grandiflora*.

Even thought, a recent study revealed that *Hibiscus sabdariffa* did not produce antibacterial activity against tested bacterial strains [ANWAR IBRAHIM *et al.*, 2014].

This might be related to the differences in the extraction methods that have been used.

In contrast, [NDUKWE *et al.*, 2005] suggested that the susceptibility of Gram–negative organisms were less than Gram–positive organisms to activity of t different plants [NDUKWE *et al.*, 2005, HUSSEIN KADHEM ABDUL *et al.*, 2007; BUTNARIU and CAUNII, 2013; RASHED and BUTNARIU, 2014b; BUTNARIU and SAMFIRA, 2012].

However, the results of the present work propose different patterns ignoring bacterial type.

S. grandiflora stem extract showed a close influence to *H. sabdariffa* at concentration of 500 mg/mL against *P. aeruginosa*.

It exhibited the second impact towards bacteria under investigation except against *S. aureus* where the *Sesbania* roots extract revealed higher effect than stems.

Even if, [KACHROO VIPIN *et al.*, 2011; BUTNARIU, 2014; IANCULOV, *et al.*, 2005] has demonstrated higher inhibition of *E. coli* and *P. aeruginosa* at 250 mg/mL.

Worth to mention the differences in the extraction could affect the results.

Conclusions

A wide range of safe alternative medications using for preventing, curing infections and to get rid of the problematic multi–resistant microbes are investigated especially from plant resources due to their effectiveness.

As conclusion from the results mentioned above, *H. sabdariffa* extract revealed an effective biological activity against G+ve and G–ve strains compared with *S. grandiflora* extracts.

The results of this work afford a high light of the potentiality of these plants in drug improvement and provide valuable information in this field.

Nevertheless, investigations are in demand to determine that these effects in vitro are achievable in vivo.

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References

1. Ahmad, R.; Ali, A.M.; Israf, D.A.; Ismail, N.H.; Shaari, K.; Lajis, N.H. Antioxidant, Radical–Scavenging, Anti–Inflammatory, Cytotoxic and Antibacterial Activities of Methanolic Extracts of Some Hedyotis Species. *Life Sciences,* **2005**, *76(17)*, p. 1953–64.
2. Anantaworasakul, P.; Klayraung, S.; Okonogi, S. Antibacterial Activities of *Sesbania Grandiflora* Extracts. *Drug Discoveries & Therapeutics,* **2011**, *5(1)*, p. 12–17.
3. Anwar Ibrahim, D.; Noman, X.; Albadani, R. Evaluation of the Potential Nephroprotective and Antimicrobial Effect of *Camellia Sinensis* Leaves versus *Hibiscus Sabdariffa* (in Vivo and in Vitro Studies). *Advances in Pharmacological Sciences,* **2014**, p. 5.
4. Bagiu, R.V.; Vlaicu, B.; Butnariu, M. Chemical Composition and in Vitro Antifungal Activity Screening of the *Allium ursinum* L. (Liliaceae). International Journal of Molecular Sciences, 2012, 13(2), p: 1426–1436.
5. Balows, A.; William, J.; Hausler, J.; Kenneth, L.; Herrmann, Henry D.

Isenberg, Shadomy, H.J. **1991**. *Manual of Clinical Microbiology,* Washington, D.C., American Society for Microbiology.
6. Bauer, A.W.; Kirby, W.M.; Sherris, J.C.; Turck, M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *American Journal of Clinical Pathology,* **1966**, *45(4)*, p. 493–6.
7. Boonkaew, T.; Camper, N.D. Biological Activities of *Ginkgo* Extracts. *Phytomedicine,* **2005**, *12(4)*, p. 318–323.
8. Burgess, N.D.; Evans, C.E.; Thomas, G.J. Vegetation Change on the Ouse Washes Wetland, England, 1972–88 and Effects on Their Conservation Importance. *Biological Conservation,* **1990**, *53(3)*, p. 173–189.
9. Butnariu, M. Detection of the polyphenolic components in *Ribes nigrum* L. *Annals of Agricultural and Environmental Medicine*, **2014**, 21(1): 11–14.
10. Butnariu, M.; Caunii, A. Design management of functional foods for quality of life improvement. *Annals of agricultural and environmental medicine*, **2013**, 20(4), 736–741.
11. Butnariu, M.; Goian, M.; Ianculov, I.; Gergen, I.; Negrea P. Studies about CO^{2+} ion influence on soy plants development and acumulation of other chemical elements (Iron, magnesium, calcium, potassium and phosphorus). *Revista de Chimie*, **2005**, 56(8), 837–841.
12. Butnariu, M.; Rodino, S.; Petrache, P.; Negoescu, C.; Butu, M. Determination and quantification of maize zeaxanthin stability, *Digest Journal of Nanomaterials and Biostructures*, **2014**, 9 (2): 745–755.
13. Butnariu, M.; Samfira, I. Free Radicals and Oxidative Stress, *Journal of Bioequivalence & Bioavailability*, **2012**, 4(3): 1.
14. Eldeen, I.M.; Elgorashi, E.E.; Van Staden, J. Antibacterial, Anti–Inflammatory, Anti–Cholinesterase and Mutagenic Effects of Extracts Obtained from Some Trees Used in South African Traditional Medicine. *Journal* of *Ethnopharmacology*, **2005**, *102(3)*, p. 457–64.
15. Elena, S.F.; Whittam, T.S.; Winkworth, C.L.; Riley, M.A.; Lenski, R.E. Genomic Divergence of Escherichia Coli Strains: Evidence for Horizontal Transfer and Variation in Mutation Rates. *International Microbiology,* **2005**, *8(4)*, p. 271–8.
16. Gellatly, S.L.; Hancock, R.E.W. Pseudomonas Aeruginosa: New Insights into Pathogenesis and Host Defenses. *Pathogens and Disease,* **2013**, *67(3)*, p. 159–173.
17. Glatthaar–Saalmuller, B.; Sacher, F.; Esperester, A. Antiviral Activity of an Extract Derived from Roots of Eleutherococcus Senticosus. *Antiviral Research,* **2001**, *50(3)*, p. 223–8.
18. Haque, N.; Chowdhury, S.A.; Nutan, M.T.; Rahman, G.M.; Rahman, K.M.; Rashid, M.A. Evaluation of Antitumor Activity of Some Medicinal Plants of Bangladesh by Potato Disk Bioassay. *Fitoterapia,* **2000**, *71(5)*, p. 547–52.
19. Harris, L.G.; Foster, S.J.; Richards, R.G. An Introduction to *Staphylococcus Aureus*, and Techniques for Identifying and Quantifying *S. Aureus* Adhesins in Relation to Adhesion to Biomaterials: Review. *European cells & materials,* **2002**, *4.* p. 39–60.
20. Higginbotham, K.L.; Burris, K.P.; Zivanovic, S.; Davidson, P.M.; Stewart, C.N., Jr. Antimicrobial Activity of *Hibiscus Sabdariffa* Aqueous Extracts against *Escherichia Coli* O157:H7 and *Staphylococcus Aureus* in a Microbiological Medium and Milk of Various Fat Concentrations. *Journal of food protection,* 2014, *77(2)*, p. 262–8.
21. Hussein Kadhem Abdul, H.; Najeh Hashem, K.; Zuhair Hamid, A. Study of the Biological Activity of Aqueous Extract of *Cuminum Cyminum* L. And *Hibiscus Sabdariffa* L. And Detection of Some Active Groups in Them. *Journal of Kerbala University,* **2007**, *5(1)*, p. 65–72.
22. Ianculov, I.; Palicica, R.; Butnariu, M.; Dumbrava.; D. Gergen, I. Obtinerea in stare cristalina a clorofilei din cetina de brad (*Abies alba*) si de pin (*Pinus sylvestris*). *Revista de Chimie–Bucharest*, **2005**, 56(4), 441–443.
23. Kachroo Vipin, Gupta K.A.; Rajesh, G. Antimicrobial Activity of *Sesbania Grandiflora* (L.) Pers. *International Research Journal of Pharmacy,* **2011**, *2(7)*, p. 85–87.
24. Kamatou, G.P.P.; Van Zyl, R.L.; Van Vuuren, S.F.; Figueiredo, A.C.; Barroso, J.G.; Pedro, L.G.; Viljoen, A.M. Seasonal Variation in Essential

Oil Composition, Oil Toxicity and the Biological Activity of Solvent Extracts of Three South African *Salvia* Species. *South African Journal of Botany,* **2008**, *74(2)*, p. 230–237.

25. Kaper, J.B.; Nataro, J.P.; Mobley, H.L.T. Pathogenic *Escherichia Coli*. *Nature Reviews Microbiology,* **2004**, *2(2)*, p. 123–140.
26. Kasture, V.S.; Deshmukh, V.K.; Chopde, C.T. Anxiolytic and Anticonvulsive Activity of *Sesbania Grandiflora* Leaves in Experimental Animals. *Phytotherapy Research,* **2002**, *16(5)*, p. 455–460.
27. Kloos, W.E.; Bannerman, T.L. Update on Clinical Significance of Coagulase–Negative Staphylococci. *Clinical Microbiology Reviews,* **1994**, *7(1)*, p. 117–140.
28. Kloos, W.E.; Musselwhite, M.S. Distribution and Persistence of *Staphylococcus* and *Micrococcus* Species and Other Aerobic Bacteria on Human Skin. *Journal of Applied Microbiology,* **1975**, *30(3)*, p. 381–5.
29. Kuete, V.; Poumale, H.M.P.; Guedem, A.N.; Shiono, Y.; Randrianasolo, R.; Ngadjui, B.T. Antimycobacterial, Antibacterial and Antifungal Activities of the Methanol Extract and Compounds from *Thecacoris Annobonae* (Euphorbiaceae). *South African Journal of Botany,* **2010**, *76(3)*, p. 536–542.
30. Lampronti, I.; Khan, M.T.H.; Bianchi, N.; Lambertini, E.; Piva, R.; Borgatti, M.; Gambari, R. Plants with Antitumor Properties: From Biologically Active Molecules to Drugs. *In:* MAHMUD, T.H.K. & ARJUMAND, A. (eds.) *Advances in Phytomedicine.* **2006**. Elsevier.
31. Lee, S.S.; Lee, E.M.; An, B.C.; Kim, T.–H.; Lee, K.S.; Cho, J.–Y.; Yoo, S.–H.; Bae, J.–S.; Chung, B.Y. Effects of Irradiation on Decolourisation and Biological Activity in Schizandra Chinensis Extracts. *Food Chemistry,* **2011**, *125(1)*, p. 214–220.
32. Lowy, F.D. Is *Staphylococcus Aureus* an Intracellular Pathogen? *Trends in Microbiology,* **2000**, *8(8)*, p. 341–343.
33. Lyczak, J.B.; Cannon, C.L.; Pier, G.B. Establishment of *Pseudomonas Aeruginosa* Infection: Lessons from a Versatile Opportunist. *Microbes and Infection,* **2000**, *2(9)*, p. 1051–60.
34. Mativandlela, S.P.N.; Lall, N.; Meyer, J.J.M. Antibacterial, Antifungal and Antitubercular Activity of (the Roots of) *Pelargonium Reniforme* (Curt) and *Pelargonium Sidoides* (Dc) (Geraniaceae) Root Extracts. *South African Journal of Botany,* **2006**, *72(2)*, p. 232–237.
35. Mesaros, N.; Nordmann, P.; Plesiat, P.; Roussel–Delvallez, M.; Van Eldere, J.; Glupczynski, Y.; Van Laethem, Y.; Jacobs, F.; Lebecque, P.; Malfroot, A.; Tulkens, P.M.; Van Bambeke, F. *Pseudomonas Aeruginosa*: Resistance and Therapeutic Options at the Turn of the New Millennium. *Clinical Microbiology and Infection*, **2007**, *13(6)*, p. 560–78.
36. Nataro, J.P.; Kaper, J.B. Diarrheagenic Escherichia Coli. *Clinical Microbiology Reviews,* **1998**, *11(1)*, p. 142–201.
37. NCCLS **1992**. Method for Determining the Bactericidal Activity of Antimicrobial Agents, Tentative Guidline M26–T. Villanova, PA, USA: National Committee for Clinical Laboratory Standards.
38. Ndukwe, K.C.; Okeke, I.N.; Lamikanra, A.; Adesina, S.K.; Aboderin, O. Antibacterial Activity of Aqueous Extracts of Selected *Chewing Sticks*. *Journal of Contemporary Dental Practice,* **2005**, *6(3)*, p. 86–94.
39. Okonogi, S.; Duangrat, C.; Anuchpreeda, S.; Tachakittirungrod, S.; Chowwanapoonpohn, S. Comparison of Antioxidant Capacities and Cytotoxicities of Certain Fruit Peels. *Food Chemistry,* **2007**, *103(3)*, p. 839–846.
40. Ozcelik, B.; Aslan, M.; Orhan, I.; Karaoglu, T. Antibacterial, Antifungal, and Antiviral Activities of the Lipophylic Extracts of *Pistacia Vera*. *Microbiological Research,* **2005**, *160(2)*, p. 159–64.
41. Palm, C.A.; Gachengo, C.N.; Delve, R.J.; Cadisch, G.; Giller, K.E. Organic Inputs for Soil Fertility Management in Tropical Agroecosystems: Application of an Organic Resource Database. *Agriculture, Ecosystems & Environment,* **2001**, *83(1–2)*, p. 27–42.
42. Panichayupakaranant, P.; Tewtrakul, S.; Yuenyongsawad, S. Antibacterial, Anti–Inflammatory and Anti–Allergic Activities of Standardised

Pomegranate Rind Extract. *Food Chemistry,* **2010**, *123(2)*, p. 400–403.
43. Prescott, L.M.; Harley, J.P.; Klein, D.A. **2002**. *Microbiology,* New York, Tata McGraw–Hill.
44. Rashed, K.N.; Butnariu, M.; Isolation and antimicrobial and antioxidant evaluation of bio-active compounds from *Eriobotrya japonica* stems, *Advanced Pharmaceutical Bulletin* **2014**, 4(1), p. 75–81.
45. Rashed, K.; Butnariu, M. Antimicrobial and Antioxidant Activities of *Bauhinia racemosa* Lam. and Chemical Content, *Iranian journal of pharmaceutical research*, **2014**, 13(3), p. 1073–1080
46. Samfira, I.; Butnariu, M.; Rodino, S.; Butu, M. Structural investigation of mistletoe plants from various hosts exhibiting diverse lignin phenotypes. *Digest Journal of Nanomaterials & Biostructures*, **2013**, 8(4), p.1679–1686.
47. Shulman, J.A.; Nahmias, A.J. Staphylococcal Infections: Clinical Aspects. *The staphylococci,* **1972**, p. 457–481.
48. Todar, K. "The Good, the Bad, and the Deadly". *SCIENCE Magazine,* **2004**, *304.* p. 1421.
49. Wilkinson, B.J. **1997**. *The Staphylococci in Human Diseases,* Churchill Livingston, London, Biology.

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