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Research Article

A PREVENTIVE APPLICATION IMPROVIZED USING ARID PLANT DERIVATES: NOSOCOMIAL PREVENTIVE REMEDY

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ABSTRACT

Objective: Epidermis acts as a niche for plethora of infection causing bacteria. It is a necessity to maintain the microbial populations in acceptable levels to attain hygienic conditions. In this study, an attempt has been made to prepare and test the functioning of antimicrobial cloth using desert plant extracts.

Methods: Comparative antibacterial and radical scavenging activity of the aqueous and methanolic extracts from plants namely *Saccharum spontaneum, Prosopsis cineria* and *Balanites aegyptica* was tested. Minimum inhibitory concentration (MIC) against *Staphylococcus aureus* MTCC 7443*, Streptococcus pneumonia* MTCC 655*, Escherichia coli* NCIM 2642*, Pseudomonas aeroginosa* MTCC 8295*, Bacillus megaterium* NCIM 2326*, Bacillus subtilis* NCIM 2329 ranged from 20 μg to 50 μg for the various extracts utilized. DNAse activity was also checked for the extracts to estimate the potency of extract for skin damage.

Results: Based on the MIC ranges, an excess amount (100 μg) of the extract was loaded on the cotton cloth and check for antimicrobial potential. DNAse activity was also checked for the extracts to estimate the potency of extract for skin damage. No extract tested was found to have DNA degradation activity.

Conclusion: Based on the manifested results, cotton cloth loaded with 100 $\mu g/cm^2$ of crude extracts from *Balanites aegyptica* may be successfully used as an antimicrobial cloth. Fabrics capable of sterilization would have potential benefits to reduce disease transfers among hospital populations and bio-warfare protection.

Keywords: *Balanites aegyptica*, Antimicrobial, Cotton cloth, Desert plant extract, Skin microflora.

INTRODUCTION

Skin, first line of defense of our body needs to be protected from various pathogens that may cause harm. Certain bacteria such as Diptheriods, *Staphylococci* sp. and fungal strains such as Malassezia constitute the normal and principle flora of the skin, their importance lies in the fact that they prevent colonization of other harmful microbes [1], but when normal flora penetrates the epidermal layer cause great damage [2,3]. Nosocomial *Staphylococcus aureus* infections and associated antibiotic resistant strains have increased over the last 20 years [4]. Similarly, occurrence of *Klebsiella* infections has been found on individuals who are using ventilators and catheters [5]. The organisms such as *S. aureus*, *Streptococcus, Escherichia coli*, *Klebsiella* sp. and *Bacillus* sp. are the most common bacteria involved in primary and secondary wound infections. The propensity of these microbes causing infections greatly increases in hospitals [6]. It thus becomes a necessity to obtain preventive hygienic condition rather than curing diseases.

Historically, Indian flora has provided an ample resource for the generation of molecules capable of controlling and curing infections. Plants have been found to contain bioactive molecules which act as pharmaceuticals and antioxidants. Some medicinal plants such as *Aloe vera* [7], *Prosopis juliflora*, and *Melaleuca alternifolia* [8] have been widely used for the treatment of skin. Interestingly, few desert plants that have been until date tested for controlling microbes causing skin diseases [9-11]. However until date, no attempt has been made to utilize the desert plants for practical application. Cotton fabric's inherent properties make it an attractive option for the microbes to grow. In addition, microbial growth tends to deteriorate the quality of fabric over time. In the current research, we have used extracts from plants growing in arid regions and adsorbed them on skin friendly cotton fabric to be used as an antimicrobial cloth. This is the first time the extracts from desert plants have been tested in conjugation with a cotton cloth to bring about applicability.

METHODS

Chemicals

Nutrient agar and broth were obtained from Himedia, Mumbai. 1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich, USA. Methanol, acetone, hexane, anthrone reagent, chloroform, sulfuric acid, sodium hydroxide, and hydrochloric acid (all reagent grade) were obtained from Rankem, New Delhi.

Sample collection and processing

Three plants namely, *Saccharum spontaneum*, *Balanites aegyptiaca* and *Prosopis cineraria* were selected for the study. Plant samples were collected during the month of February from within and around the campus of Amity University Rajasthan, Jaipur. Specifically, stem of *S. spontaneum*, fruit coat of *B. aegyptiaca* and leaves of *P. cineraria* were used for this study. Briefly, stem of *S. spontaneum* was cut into 12-inch pieces and allowed to dry at 45°C for 48 hrs in hot air oven. After drying, the sample was ground to powder and passed through sieve (minimum inhibitory concentrations [MIC] 425). Similarly, fruit coat of *B. aegyptiaca* and leaves of *P. cineraria* were also processed.

Solvent extraction and preliminary identification of secondary metabolites

Soxhlet extraction of the 30 g of powdered samples, enveloped in Whatmann filter paper No. 1, was performed. 300 ml of methanol and distilled water each were used for the extraction separately. Extract obtained was dried and stored for further processing. The extract was re-suspended to form a stock of 1 mg/ml and was checked for the presence of bioactive molecules [12,13] including alkaloids, tannins,

glycosides [14] saponins [15], terpenes, flavonoids [16], steroids, quinones, and coumarins [17,18].

Culture and maintenance of microorganisms

Pure cultures (*S. aureus* MTCC 7443, *S. pneumonia* MTCC 655, *E. coli* NCIM 2642, *Pseudomonas aeroginosa* MTCC 8295, *Bacillus megaterium* NCIM 2326, *Bacillus subtilis* NCIM 2329) of all experimental microbes were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh and National Chemical Laboratory, Pune. The pure microbial cultures were maintained on agar medium and further maintained by sub-culturing regularly on the same medium and stored at 4°C before use in experiments.

Determination of MIC

MIC testing was performed by a serial dilution technique whereby sterilized paper discs were taken and impregnated with extracts at different concentrations. The microbial plates were incubated for 24 hrs at 27°C. The lowest concentrations without visible growth were defined as MICs. The control that was used, i.e., ampicillin had 10 µg/disc.

Adsorption of extract on the cotton cloth

The crude extracts thus prepared, were filtered using syringe filter (0.45 µ). Cotton cloth was obtained from the local market and rinsed several times in normal water, followed by distilled water and then autoclaved. This was subsequently cut into 1 cm^2 pieces and then autoclaved again. These pieces were dried under the laminar air flow. These sterilized cotton pieces were dipped using autoclaved forceps into the samples of fixed concentration which were greater than the corresponding MIC (100 µg). They were then dried and stored for further processing.

Activity testing for the antimicrobial cloth

Activity testing for the cloth was performed following protocol AATCC Test Method 147 [19]. The organisms used for testing were *B. megaterium, B. subtilis, E. coli, P. aeruginosa, S. aureus,* and *S. pneumonia*. The antimicrobial activity of the impregnated cloth was also checked after rinsing the cloth in distilled water.

Determination of antioxidant activity

DPPH as a radical scavenger was used to measure the antioxidant efficacy of reconstituted samples. Normally, DPPH has absorbance maxima at around 517 nm, which diminishes in the presence of reducing agent. The change in color indicates the strength of the anti-oxidative potential of the molecule being tested [20]. The DPPH solution (0.25 mM) was prepared in 95% methanol. Ascorbic acid was used as a reference standard and dissolved in DDW to make the stock solution. The free radical scavenging activity (RSA) of metabolic extracts was measured in terms of hydrogen donating or RSA using the stable radical DPPH using the following equation:

$$\text{DPPH scavenging effect (\%)} = [A_O - A_T]/Ao \times 100$$

Where, A_o and A_T are absorbance of control and the sample at 517 nm. [21].

DNAse assay

It has been shown that some secondary metabolites have a tendency to be adsorbed through skin. The antimicrobial cloth being tested should not have any negative effect on the skin and the cells lying beneath the epidermis. Hence, the probable presence of DNase activity was checked for the reconstituted samples (1 mg/ml concentration) from different plant. Briefly, 20 µg of the extracts were incubated with 10 µg of calf thymus DNA at room temperature for 1 hr. After incubation, the reaction mixture was monitored on 0.8% agarose gel to check for degradation of DNA.

RESULTS

Extraction and preliminary identification of secondary metabolites

Plant material was dried, ground, and processed for secondary metabolite extraction. In general, the seeds of *B. aegyptica* have been used to extract oil for commercial purposes, but the seed coat is a by-product of the industry. Hence, an attempt has been made to utilize the unused part of the plant for the preparation of the antimicrobial cloth. The methanolic and aqueous extract of the samples were obtained using a soxhlet device. Clarity of the extraction chamber indicating complete extraction was obtained after 9-10 cycles in the soxhlet. The extract obtained was dried in an evaporator to obtain concentrated exudes from various sources. The dry weight of the methanolic and aqueous extracts obtained from *S. spontaneum* was 1.679 g and 1.06 g, *B. aegyptica* was 11.65 g and 13.234 g and *P. cineraria* was 1.895 g and 1.731 g, respectively from 30 g of each plant material. The dried extract was re-suspended in autoclaved distilled water to obtain stock solution of 1 mg/ml. Numerous classes of metabolites were detected in the extracts as presented in Table 1.

Minimum inhibitory activity

The antimicrobial activity of the extracts were studied in different concentrations (10, 20, 30, 50,100, and 250 µg/ml) against six bacterial strains including four Gram-positive and two Gram-negative. *Bacillus subtillis* and *E. coli* were more sensitive to the aqueous extracts of the *S. spontaneum. S. spontaneum* extract contained quinine, tannins and alkaloids (Table 1). The inhibition zone ranged from 5 mm to 10 mm for all the sensitive bacteria on adsorbed cloth. The corresponding MIC is mentioned in Table 2.

RSA of the samples

The free RSA of the extracts were calculated and the results obtained were as indicated in Fig. 1a. Maximum RSA (that is, 87.04%) was found to be in the methanolic extract of *B. aeyptiaca* followed by the extract from *S. spontaneum*. On comparison of the RSA of aqueous extracts, it was found *B. aeyptiaca* indicated maximal RSA activity. The aqueous extracts tend to show less RSA because of the presence of tannins.

DNAse activity assay

The maximum concentration which has been loaded is 100 ug. Based on the facts, the maximum quantity which could be adsorbed is approximately 17 µg. Keeping these concentrations in mind, an amount of 20 µg was incubated with 10 µg of genomic DNA sample. The presence of DNAse activity in any of the extract would result in degradation of the test DNA sample. DNA and the extract sample after an incubation period of one hour was loaded on 0.8% gel for detection of DNA degradation.

It was observed none of the extract gave a visible degradative activity (Fig. 1b).

Antimicrobial action of finished fabric

Antimicrobial activity (modified AATCC 147) was tested for the treated and untreated cloth. *B. aegeptica* has indicated an increased activity

Table 1: Phytochemical constituents of the plant extracts

Compounds	*S. spontaneum*		*B. aegyptiaca*		*P. cineraria*	
	H_2O	MeOH	H_2O	MeOH	H_2O	MeOH
Quinine	+	-	+	-	+	-
Terpenes	-	+	-	-	-	-
Alkaloids	+	-	-	-	+	+
Saponins	-	+	+	+	-	-
Tannins	+	-	+	-	+	+
Carbohydrates						
Reducing sugars	+	+	+	+	+	-
Starch	-	-	-	-	-	-
Coumarins	-	+	-	-	-	-
Steroids	-	-	-	-	-	-
Glycosides	-	+	-	-	-	+
Proteins	-	-	-	-	-	-
Flavanoids	-	-	-	-	-	-

+: Presence of metabolite, -: Absence, H_2O: Water, MeOH: Methanol, *S. spontaneum: Saccharum spontaneum, B. aegyptiaca: Balanites aegyptiaca, P. cineraria: Prosopis cineraria*

Table 2: MIC of the extracts for Gram-negative and Gram-positive bacteria

Sample	*B. subtillis* (in μg)	*E. coli* (in μg)	*B. megaterium* (in μg)	*P. aeruginosa* (in μg)	*S. aureus* (in μg)	*S. pneumonia* (in μg)
S. spontaneum						
MeOH	20	20	30	20	20	30
Aq	20	20	30	50	50	30
P. cinera						
MeOH	40	40	40	40	40	40
Aq	40	40	40	30	50	30
B. aegyptiaca						
MeOH	50	50	50	50	50	50
Aq	40	50	50	50	50	40

S. spontaneum: Saccharum spontaneum, B. aegyptiaca: Balanites aegyptiaca, P. cineraria: Prosopis cineraria, B. subtillis: Bacillus subtillis, MeOH: Methanol, MIC: Minimum inhibitory concentration

especially, toward *S. pneumonia* and *P. aeruginosa*. A standard amount of 100 μg of extract was loaded on each cloth of 1 cm^2. Untreated cloth indicated no antimicrobial activity. Treated cloth showed different degrees of the zone of inhibition for the different bacteria. Water extract of *P. cineraria* was found to be least effective when loaded on cotton cloth. In comparison to all other extracts, the antimicrobial activity of the *B. aegeptica* extracts gave better results (Fig. 2).

DISCUSSION

Selected plants were taken and subjected to processing for extraction of the active fractions, namely the aqueous and alcoholic extract. Based on our experimentation, *P. aeruginosa* and *S. pneumonia* were found to be more susceptible to the extracts from *P. cineraria. Prosopis* sp. has been shown to contain many bioactive molecules [22] demonstrating antimicrobial activity. The aqueous extract of *P. cineraria* contains quinones and alkaloids (Table 1) which may be regulating the inhibition of these two bacteria. The presence of piperidine alkaloids in *P. juliflora* has been attributed for antibacterial activity [23]. Many alkaloids have been found to inhibit Pseudomonas sp. by affecting their quorum sensing [24]. A number of explanations can be given for the difference in biological activity reports of some common extracts against same or similar microorganism, but the first logic is dissimilarities in phytochemicals of plants.

As per earlier citations [25], methanolic extract of *B. aegyptiaca* revealed potential antioxidant activity but antibacterial activity was found to be relatively lower to that of the standard antibiotics used. In comparison, we found that although the antibacterial activity was comparable to the standards but, in *S. pneumoniae* and *P. aeruginosa*, the inhibition zone was found to be particularly higher. Very few metabolites have been found to inhibit *P. aeruginosa* because of it property to form biofilm. Previous experiments have also confirmed that Balanites is effective against Pseudomonas [26].

Tannins are prone to auto-oxidation in air, resulting in the release of hydrogen peroxide, thus affecting the antioxidant potential of extracts [27]. Previous records indicate that Balanites has good antioxidant potential [28,29], hence the RSA can be observed in the water extract of Balanites also.

The plant extracts tend to contain many metabolites which may have a tendency to get adsorbed through the skin [30]. Studies have shown that dermal absorption of metabolites can go to a maximum limit of 17% of topical applied dose [31,32]. On the basis of results obtained (Fig. 1a), it has been assumed that the antimicrobial cloth designed using methanolic extracts of all samples and water extract of Balanites should be safe on skin because of good RSA for each of the extracts.

DNase activity was also tested to assess the possible DNA damaging activity of the extract. Previously, it has been indicated that petroleum ether extract of Balanites had DNA fragmentation ability [33], but no such activity was observed in the methanolic and aqueous extract in our experiments hence, it was deduced that none of the samples used

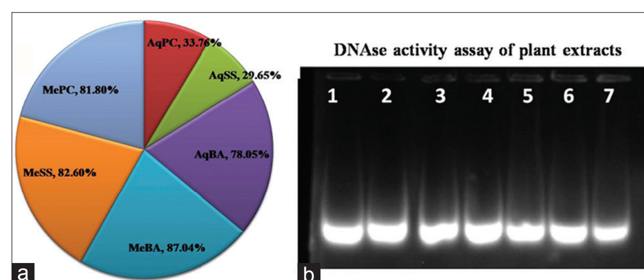

Fig. 1: (a) Percentage radical scavenging activity of the extracts obtained (b) Agarose gel electrophoresis of the samples 1: Control; 2: AqBA; 3: AqPC; 4: AqSS; 5: MeBA; 6: MePC; 7: MeSS (BA: *Balanites aegyptiaca*, PC: *Prosopis cineraria*, SS: *Saccharum spontaneum*, Aq: Aqeous, Me: Methanolic)

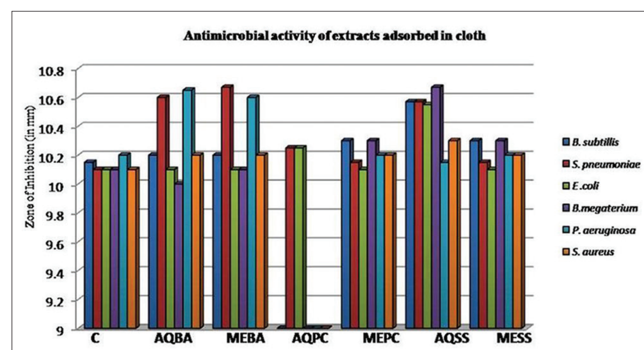

Fig. 2: Zone of inhibition observed for the antimicrobial cloth. (C: Control; BA: *Balanites aegyptiaca*; PC: *Prosopis cineraria*; SS: *Saccharum spontaneum*; Aq: Aqeous; Me: Methanolic)

will have negative effect of the skin where the antimicrobial cloth remains in contact. Consequently from the above results, it is clear that the samples do not have any DNAse activity, i.e., DNA degradation activity hence the development of an antimicrobial cloth and its usage will not have adverse effect on the human skin.

Balanites is a rich source of saponins and hence, a better inhibitory potential can be attributed to the presence of saponins. The antimicrobial cloth made using aqueous extract of *S. sponteneum* has also showed good antimicrobial potential. Interestingly, quinones have been found to be an active component in the water extracts and all the aqueous extracts have shown higher inhibitory activity toward *S. pneumonia* and *E. coli*. It has been found that quinones tend to disrupt the activity of NADH dehydrogenases in microbes and thus their survival [34].

Hardly, 10% of the antimicrobial activity was retained after a single wash. Reusability of the antimicrobial cloth need to be enhanced using improved techniques for better applicability.

CONCLUSION

It has been noted, that all the three plants have proved to be source of effective antimicrobials [35-37]. It was found that when adsorbed on cloth, the antimicrobial affectivity varied. Antimicrobial activity of the extracts obtained from *B. aegeptica* and *S. sponteneum* has given promising inhibitory action as compared to other extracts. However comparatively the RSA activity of the *B. aegeptica* is better than *S. spontaeneum*. The mechanism of the *S. spontaeneum* activity may be due to the presence of free radicals, and hence continuous contact with the skin may damage the skin. Hence, based on our experimentation, an antimicrobial cotton cloth loaded with 100 μg/cm^2 of extract can be safely applied for human utility.

REFERENCES

1. MacLachlan A. Body Bacteria: Exploring the Skin's Microbial Metropolis. USA: FINDINGS National Institute of General Medical Sciences; 2012. p. 1-9.
2. Nester E, Anderson D Jr, Roberts CE. Evans Microbiology: A Human Perspective. New York: McGraw-Hill Higher Education; 2011.
3. Dellion S, Morel P, Vignon-Pennamen D, Felten A. Erythrasma owing to an unusual pathogen. Arch Dermatol 1996;132:716-7.
4. Baorto EP, Baorto D. *Staphylococcus Aureus* infection. Medscape Med News. Available from: http://www.medscape.com/viewarticle/971358.
5. Lynch JP 3rd, Zhanel GG. *Streptococcus pneumoniae*: Epidemiology and risk factors, evolution of antimicrobial resistance, and impact of vaccines. Curr Opin Pulm Med 2010;16:217-25.
6. Siegel JD, Rhinehart E, Jackson M, Chiarello L. HICPAC, Management of Multidrug-Resistant Organisms in Healthcare Settings 2006. Available from: http://www.cdc.gov/ncidod/dhqp/pdf/ar/mdroGuideline2006.pdf.
7. Aly R. Microbial Infections of Skin and Nails. Medical Microbiology. 4th ed. Galveston: University of Texas Medical Branch; 1996.
8. Jia Y, Zhao G, Jia J. Preliminary evaluation: The effects of *Aloe ferox* Miller and *Aloe arborescens* Miller on wound healing. J Ethnopharmacol 2008 20;120(2):181-9.
9. Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia* (Tea Tree) oil: A review of antimicrobial and other medicinal properties. Clin Microbiol Rev 2006;19(1):50-62.
10. Ahmad M, Ghafoor N, Aamir MN. Antibacterial activity of mother tinctures of cholistan desert plants in Pakistan. Indian J Pharm Sci 2012;74(5):465-8.
11. McKay DL, Blumberg JB. A review of the bioactivity of South African herbal teas: Rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*). Phytother Res 2007;21(1):1-16.
12. Said O, Khalil K, Fulder S, Azaizeh H. Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. J Ethnopharmacol 2002;83:251-65.
13. Suleiman MN. The *in vitro* phytochemical investigation on five medicinal plants in Anyigba and its environs, Kogi State, Nigeria. Der Pharm Sin 2011;2:108-11.
14. Joshi N, Bhatt S, Dhyani S, Nain J. Phytochemical screening of secondary metabolites of *Argemone mexicana* Linn. flowers. Int J Curr Pharm Res 2013;5:144-7.
15. Treare GE, Evans WC. Pharmacognosy. 17th ed. London: Bahiv Tinal; 1985.
16. Kokate C. Practical Pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan; 1994.
17. Khandelwal KR. Practical Pharmacognosy, Techniques and Experiments. 12th ed. Pune, India: Nirali Prakashan; 2004. p. 157.
18. Ghani A. Medicinal Plants of Bangladesh: Chemical Constituents and Uses. Dhaka: The Asiatic Society of Bangladesh; 2003. p. 500-8.
19. Geethadevi R, Maheshwari V. Imparting antimicrobial finish on selected regenerated cellulosic fabrics with herbal oil combinations. J Textile Sci Eng 2013;4:144.
20. Song C, Yu H, Zhang M, Yang Y, Zhang G. Physicochemical properties and antioxidant activity of chitosan from the blowfly *Chrysomya megacephala* larvae. Int J Biol Macromol 2013;60:347-54.
21. Subhashini N, Nagarajan G, Kavimani S. *In vitro* antioxidant and anticholinesterase activities of *Garcinia combogia*. Int J Pharm Pharm Sci 2011;3:129-32.
22. Vedak S, Raut SV. Study on antibacterial compounds from methanolic extract of bark of *Prosopis juliflora* [Vilayati babhul]. Int J Pharm Sci Business Manag 2014;2:1-14.
23. Sathiya M, Muthuchelian K. Investigation of phytochemical and antibacterial potential of ethanolic leaf extract of *Prosopis julilfora* DC. Ethanolbaotonical leaflets. 2008;12:1240-5.
24. Ahmad A, Khan KA, Ahmad VU, Qazi S. Antibacterial activity of juliflorine isolated from *Prosopis juliflora*. Planta Med 1986;(4):285-8.
25. Park J, Kaufmann GF, Bowen JP, Arbiser JL, Janda KD. Solenopsin A, a venom alkaloid from the fire ant *Solenopsis invicta*, inhibits quorum-sensing signaling in *Pseudomonas aeruginosa*. J Infect Dis 2008;198(8):1198-201.
26. Kahsay T, Mulugeta A, Unnithan CR. Antioxidant and antibacterial activities of *Balanites aegyptiaca* delil from Northern Ethiopia. Am J PharmTech Res 2014;4(3):415-22.
27. Shahid M, Jahan N, Shahzad A, Sahai A, Sharma S, Parveen S. Antimicrobial potential of *Balanites Aegyptiaca* [L.] Del, Stevia Rebaudiana [Bert.]Bertoni, *Tylophora Indica* [Burm.f.] Merrill, and *Cassia Sophera* [Linn.]. Open Conf Proc J 2012;3:63-9.
28. Smith AH, Imlay JA, Mackie RI. Increasing the oxidative stress response allows *Escherichia coli* to overcome inhibitory effects of condensed tannins. Appl Environ Microbiol 2003;69:3406-11.
29. Sen S, Chakraborty R. The Role of Antioxidants in Human Health, Oxidative Stress: Diagnostics, Prevention, and Therapy, ACS Symposium Series. p. 201, 1-37.
30. Olila D, Opuda-Asibo J, Olwa-Odyek. Bioassay-guided studies on the cytotoxic and *in vitro* trypanocidal activities of a sesquiterpene (Muzigadial) derived from a Ugandan medicinal plant (*Warburgia ugandensis*). Afr Health Sci 2001;1(1):12-5.
31. Robbins PJ, Cherniack MB. Review of the bio distribution and toxicity of the insect repellent N, N-diethyl-m-toluamide [DEET]. J Toxicol Environ Health 1986;18:502-25.
32. Feldmann RJ, Maibach HI. Absorption of some organic compounds through the skin in man. J Invest Dermatol 1970;54:399-404.
33. Al-Ghannam SM, Ahmed HH, Zein N, Zahran F. Antitumor activity of balanitoside extracted from *Balanites aegyptiaca* fruit. J Appl Pharm Sci 2013;3:179-91.
34. Imlay J, Fridovich I. Exogenous quinones directly inhibit the respiratory NADH dehydrogenase in *Escherichia coli.* Arch Biochem Biophys 1992;296:337-46.
35. Ripa FA, Haque M, Imran-Ul-Haque M. *In vitro* antimicrobial, cytotoxic and antioxidant activity of flower extract of *Saccharum Spontaneum* Linn. Eur J Sci Res 2009;30:478.
36. Hartwell JL. Plants used against cancer: A survey. Lloydia 1971;2:30-4.
37. Swapnil SS, Verma SK. Antibacterial properties of alkaloid rich fractions obtained from various parts of *Prosopis juliflora.* Int J Pharm Sci Res 2011;2:114-20.