The Pharma Innovation Journal 2019; 8(1): 01-05

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(1): 01-05

www.thepharmajournal.com
Received: 01-11-2018
Accepted: 03-12-2018

Minakshi Rajput
Department of Botany and Microbiology, Gurukul Kangri University, Haridwar, Uttarakhand, India

Navneet
Department of Botany and Microbiology, Gurukul Kangri University, Haridwar, Uttarakhand, India

Correspondence
Minakshi Rajput
Department of Botany and Microbiology, Gurukul Kangri University, Haridwar, Uttarakhand, India

Antimicrobial potential of *Hydnocarpus laurifolia* seeds utilized in folkloric medicine: A possible alternative in the treatment of scalp infections

Minakshi Rajput and Navneet

Abstract
Infections related to hairs and scalp are one of the major current concerns as they are the main reason of hair damage and loss. In this behalf, many antimicrobial drugs available in market suffer from the resistance of pathogenic strains. Antimicrobial resistance stems from a number of factors, including inappropriate and excessive use of antimicrobial drugs. Therefore, the need for new antimicrobials have been dramatically increasing and medicinal plants are considered as one of the most promising sources for the discovery of new antimicrobial compounds. In this context, the current investigation aimed to evaluate the antimicrobial activity, MIC, and MBC/MFC of different seed extracts of *Hydnocarpus laurifolia*. Four different organic solvents including petroleum ether, ethyl acetate, methanol, and water were used to prepare the crude extracts. Antimicrobial activity and MIC of the extracts were determined by agar well diffusion method and broth microdilution method respectively. Among all the four solvents, ethyl acetate extract exhibited best antimicrobial potency against all the five selected pathogens i.e. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Microsporum audouinii*, *Malassezia furfur*, and *Candida albicans* with the zone of inhibitions ranging from 12.33±0.33 to 33.16±0.88 mm followed by methanol (10.33±0.33 to 19.33±0.33 mm) and petroleum ether (09.00±0.00 to 16.33±0.60 mm). Water extract did not show much potential against all the tested pathogens. MICs of the extracts ranged from 3.12-25 mg/ml for ethyl acetate, 12.5-100 mg/ml for petroleum ether and 6.25-100 mg/ml for methanol extract. MBCs of the different extracts ranged from 6.25-50 mg/ml for ethyl acetate, 50-200 mg/ml for petroleum ether and 12.5-200 mg/ml for methanol. Hence, from the results it can be confirmed that ethyl acetate extract showed lowest MICs and MBCs/MFCs values for all the tested microbial strains and zone of inhibitions greater than reference antimicrobial drugs. Therefore it can be concluded that organic crude extracts could be used as potential sources of antimicrobial agents other than conventional antibiotics or antifungals and they have perspective for further more inclusive studies.

Keywords: Scalp infections, *Hydnocarpus laurifolia*, antimicrobial activity, MIC, MBC/MFC

1. Introduction
Bacterial and fungal scalp infections are considered one of the most common issues of hair damage and loss. In daily routine life, scalp and hairs regularly come into contact with combs, fingers, styling implements, hats etc. which may introduce microbial infections and infestations. Scalp surface is very prone to the superficial mycotic and bacterial infections which may produce different types of scalp conditions such as dandruff, seborrheic dermatitis, folliculitis, and tinea capitis etc. [1, 2].

Nowadays, even though allopathic medicines are readily available and potentially effective in curing various ailments, the trend is again shifting towards the traditional folk medicines as people recognize that allopathic medicines are expensive than herbal and have various adverse effects on the human body such as toxicity and the emergence of resistant strains [3, 4, 5]. The situation is alarming in many developing as well as developed countries due to indiscriminate use of various antimicrobials. There is a drastic increase in the Multiple Drug Resistant strains of pathogenic microbes at the present time, due to which the efficacy of many traditional antibiotics are decreasing and consequently the frequency of therapeutic failures are increasing [6]. Hence, there is urgent continuous need for new alternative antimicrobial substances having novel chemical formulas and diverse mechanisms of actions against re-emerging pathogenic microbes. Medicinal plants are now gaining much attention as a source of new inexpensive antimicrobial compounds as they have negligible side effects, toxicity and an enormous therapeutic potential for the treatment of various infectious diseases and this is one of the possible ways of rebating the burden of microbial resistance [7, 8, 9, 10].

It has been clinically proven that herbal formulations enhance the growth of hair, strengthen and nourish hair and scalp and stop hair fall [11].

Hydnocarpus laurifolia (Dennst) Sleummer commonly known as Chaulmoogra or Tuvaraka and synonymously known as *Hydnocarpus pentandra* (Buch-Ham) Oken. and *Hydnocarpus wightiana* Blume. *Hydnocarpus* belongs to family Flacourtiaceae and diversely found in tropical forests of Ghats, along with the coast of Maharashtra to Kerala, Assam, Tripura, Sri Lanka and widely cultivated in Southeast Asia, mainly in China, Indonesia, Taiwan, Thailand, and Malaysia [12].

This plant grows up to the height of 50 feet. Seeds of *H. laurifolia* are a potential source of oil known as Chaulmoogra oil. This oil is utilized traditionally in the treatment of leprosy, constipation, hemorrhoids, itching, cervical lymphadenitis, ophthalmia, fever, worm infestation, inflammation, chronic skin infections and for healing wounds and ulcers. It is also used for its anti-obesity activity and effectively applied for the treatment of rheumatism, bruises, sprains, sciatica and chest infections [13]. The seeds of *H. laurifolia* possess high medicinal potential as they contain an array of therapeutically significant groups of phytoconstituents such as triterpenoids, aglycones, flavones, glycosides and a wide range of fatty acids and esters. Extracts of seed contain compounds structurally related to the flavonolignans namely hydnocarpin, hydnowightin, neohydnocarpin, luteolin, and isohydnocarpin [14].

This study was encouraged by early reported medicinal properties of *H. laurifolia* as it is widely utilized in several traditional herbal formulations. The motive of the present investigation is to find out new prospectives of this plant in the treatment of scalp related infections. This piece of work mainly focuses on the *in vitro* antimicrobial potential of different seed extracts of *H. laurifolia* and to determine the minimum inhibitory and bactericidal/fungicidal concentrations against selected microbial scalp pathogens.

2. Material and Methods

2.1 Chemicals and Reagents

The chemicals and drugs used in the present investigation such as Erythromycin, Bacitracin, Neomycin, Ketoconazole and Mueller Hinton Agar were purchased from HiMedia, Mumbai, India. Terbinafine (Tyza) and Griseofulvin (Grisovin) tablets were obtained from Abbott Healthcare, Mumbai, India and GlaxoSmithKline Pharmaceuticals Ltd., Bangalore, India respectively. Ascorbic acid was obtained from Sisco Research Laboratories, Mumbai, India. Petroleum ether (PET) and Ethyl acetate (EA) were purchased from CDH (Central Drug House), New Delhi, India. Methanol (MeOH) was purchased from Merck Life Science, Mumbai, India, and DMSO (Dimethly Sulphoxide) from Rankem, Avantor Performance Materials, Maharashtra, India. All these and other chemicals/reagents used in this research work were of analytical grade and utilized without further purification.

2.2 Collection and Identification of Plant Material

Seeds of *H. laurifolia* were collected from Haridwar, Uttarakhand, India between the months of February and March 2017 and packed immediately after picking and stored until processed. Plant specimen was identified and authenticated by BSI (Botanical Survey of India) Dehradun, Uttarakhand, India on the basis of morphological features of the plant and by the help of already present database. A voucher specimen (Accession No. 118139) was prepared and deposited in the herbarium of BSI, Dehradun for future reference.

2.3 Preparation of Extracts

The seeds of *H. laurifolia* were rinsed with fresh tap water followed by distilled water to remove the dirt on the surface and air dried to remove the moisture content. The dried seeds were crushed to small pieces using pestle and mortar then coarsely powdered with an electric grinder. For the preparation of different seed extracts, 200 g of seed powder was sequentially extracted by immersing with 600 ml of four solvents namely petroleum ether, ethyl acetate, methanol and distilled water using Soxhlet extractor for 3 days through successive method. After complete extraction, the crude extract was obtained by filtering with filter paper (Whatman No. 41) and the solvent was evaporated through vacuum rotary evaporator and stored in sterile vials at 4°C until further use. For antimicrobial testing, all the extracts were dissolved in dimethyl sulfoxide (DMSO) to prepare a final concentration of 200 mg/ml. Extract yield was expressed as:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dry extract (g)}}{\text{Weight of the sample used for the extraction (g)}} \times 100$$

2.4 Test Microorganisms

Bacterial strains *Pseudomonas aeruginosa* (MTCC 2474), *Staphylococcus aureus* (MTCC 1144) causing scalp folliculitis, dermatophyte *Microsporum audouinii* (MTCC 8197) causing tinea capitis, yeast *Malassezia furfur* (MTCC 1374) and *Candida albicans* (MTCC 227) associated with dandruff and seborrheic dermatitis were used for this study. All the microbial strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh.

2.5 Preparation of Inoculums

Stock cultures of all the pathogens were maintained at 4°C on the slants. Active cultures for experiments were prepared by transferring cells from stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria, Sabouraud Dextrose broth (SDB) for *M. audouinii*, *C. albicans* and Emmons modified Sabouraud Dextrose broth for *M. furfur*. Bacterial strains were incubated for 24 hrs at 37 °C and fungal strains were incubated at 30°C for 7 days.

2.6 Antimicrobial Assay

The antimicrobial activity of the extracts was evaluated by Agar Well Diffusion assay [15]. The turbidity of the inoculum was adjusted to 1.5×10^8 CFU/ml (corresponding to 0.5 McFarland standards). The antibacterial efficacy of the plant extracts was compared by three reference standard antibiotics (erythromycin, bacitracin, and neomycin) and similarly the antifungal activity was compared with three reference antifungal drugs (terbinafine, griseofulvin and ketoconazole). The final concentrations for the antimicrobial drugs were erythromycin (15 µg/ml), bacitracin (8 units), neomycin (10 µg/ml), terbinafine (1 µg/ml), griseofulvin (10 µg/ml) and ketoconazole (10 µg/ml). Erythromycin, bacitracin and neomycin were thoroughly dissolved in distilled water and terbinafine, griseofulvin and ketoconazole were properly mixed in DMSO to prepare the final concentration for the antimicrobial susceptibility testing. DMSO and distilled water were the negative control. Antimicrobial activity was

investigated by using Mueller-Hinton Agar for both the bacterial strains, Sabouraud Dextrose Agar for *M. audouinii*, *C. albicans* and Emmons modified Sabouraud Dextrose Agar for the yeast *M. furfur*. 45 µl of the final concentration of the plant extracts and controls were dispensed into the wells of 6 mm diameter.

2.7 Determination of MIC, MBC and MFC Values

Minimum inhibitory concentrations (MIC) of crude extracts of *H. laurifolia* were determined by broth microdilution method [16, 17, 18, 19]. Stock solutions were prepared with the concentration of 200 mg of crude plant extracts in 1 ml of DMSO and further diluted using two-fold serial dilution in sterile broth. This provides the series of test concentrations of 200, 100, 50, 25, 12.5, 6.25 and 3.13 mg/ml respectively. The inoculums of test strains were prepared from the fresh cultures.100 µl volume of samples were poured into the well of 96-well microtiter plate. Then after a volume of 100 µl of test strain broths were added to them. After the incubation, the smallest concentration of the test sample that inhibited the growth of the test organism was considered as MIC of the plant extract. All the dilutions of crude extracts were also cultured in agar media for sterility test. Minimum bactericidal/fungicidal concentrations (MBC/MFC) of the plant crude extracts were determined by sub-culturing the samples from the wells of MIC microtiter plate with no visible growth on freshly prepared agar medium. The smallest concentration of crude extract with the absence of visible growth on the agar plate after the incubation was taken as MBC/MFC.

2.8 Statistical analysis

All the *in vitro* experiments were performed in triplicate and the experimental data was expressed as mean ± SE (Standard error). Results were also analyzed statistically through One-way analysis of variance (ANOVA) and differences among the means were determined for significance at $P \leq 0.05$ using Microsoft Excel 2013.

3. Results

3.1 Extraction Yield

The percent yield of the extraction, color, and consistency of the crude extracts of *H. laurifolia* are given in Table 1. It was observed that ethyl acetate extract gave a maximum yield of phytochemicals about 34.81%, followed by petroleum ether (19.34%), water (7.74%) and methanol (6.49%).

Table 1: Extraction yield, color and consistency of the seed extracts of *H. laurifolia*

Extracts	Extraction Yield (%)	Color	Consistency
Petroleum ether	19.34	Cream	Liquid (Oil)
Ethyl acetate	34.81	Brown	Gummy
Methanol	6.49	Brown	Semi solid
Water	7.74	Brown	Semi solid

3.2 Antimicrobial Assay

In vitro antibacterial and antifungal activity of crude seed extracts against bacterial, fungal and yeast strains were quantitatively assessed by investigating the presence or absence of inhibition zones. The results of the antimicrobial screening of the extracts and reference drugs were summarized in Table 2. Data showed the huge antimicrobial activity variation among different extracts. It was observed that *P. aeruginosa* was the most sensitive strain among all the five tested pathogens. All the four seed extracts showed the zone of inhibitions against *P. aeruginosa* ranging from 9.33±0.44 to 33.16±0.88 mm and ethyl acetate was the most effective extract with 33.16±0.88 mm zone of inhibition followed by methanol, petroleum ether, and water. In the case of *S. aureus*, the diameter of inhibition zones ranging from 10.33±0.33 to 21.00±0.86 mm and ethyl acetate extract was the most potent extract with 21.00±0.86 mm inhibition zone followed by petroleum ether and methanol. It was observed that ethyl acetate extract exhibited moderate anti-yeast activity against *C. albicans* with 12.33±0.33 mm zone of inhibition followed by methanol and petroleum ether. Furthermore, against the dandruff yeast *M. furfur* only ethyl acetate extract showed remarkable activity with 15.16±0.72 mm inhibition zone and no other extracts exhibited any activity under tested concentration. Dermatophyte *M. audouinii* was most sensitive to ethyl acetate followed by methanol and petroleum ether with the zone of inhibition ranging between 11.26±0.39 to 12.83±0.16 mm. DMSO solvent was used as negative control which did not show any antimicrobial potential. Reference antibiotics also tested for bacterial sensitivity and in the case of *S. aureus* it was observed that erythromycin showed the maximum zone of inhibition which was 20.33±0.33 mm followed by neomycin and bacitracin. *P. aeruginosa* was most sensitive to neomycin (21.43±0.29 mm) followed by erythromycin and bacitracin. To check the sensitivity of fungal strains, reference antifungals were utilized and it was investigated that all the tested fungal strains were resistant to the tested concentration of griseofulvin. *M. audouinii* was most sensitive to terbinafine with 55.16±0.60 mm zone of inhibition and *M. furfur* was most sensitive to ketoconazole with an inhibition zone of 15.40±0.30 mm.

Table 2: Zone of inhibitions of seed extracts of *H. laurifolia* and reference antimicrobials against five tested pathogens

Extract/ Standard	*Inhibition Zone Diameter (mm)				
	Bacterial Strains		Fungal Strains		
	S. aureus	*P. aeruginosa*	*C. albicans*	*M. furfur*	*M. audouinii*
Petroleum ether	16.33±0.60	11.16±0.16	09.00±0.00	-	11.26±0.39
Ethyl acetate	21.00±0.86	33.16±0.88	12.33±0.33	15.16±0.72	21.16±0.60
Methanol	10.33±0.33	19.33±0.33	10.33±0.33	-	12.83±0.16
Water	-	09.33±0.44	-	-	-
Erythromycin	20.33±0.33	18.23±0.14	NT	NT	NT
Neomycin	18.50±0.28	21.43±0.29	NT	NT	NT
Bacitracin	11.83±0.44	17.73±0.37	NT	NT	NT

Terbinafine	NT	NT	-	10.50±0.28	55.16±0.60
Griseofulvin	NT	NT	-	-	-
Ketoconazole	NT	NT	20.76±0.39	15.40±0.30	25.56±0.34
DMSO (Negative Control)	-	-	-	-	-

* Values are the mean of three replicates; - : No zone of inhibition; NT: Not tested; Diameter of well: 6 mm; $P \leq 0.05$

3.3 MIC, MBC/MFC

The MIC and the MBC/MFC of all the four seed extracts are shown in Fig.1. The MIC of seed extracts ranged from 6.25-100 mg/ml for *S. aureus*, 3.12-100 mg/ml for *P. aeruginosa*, 25-100 mg/ml for *C. albicans*, 6.25-50 mg/ml for *M. audouinii* and 12.5 mg/ml for *M. furfur*. The MBC/MFC values of extracts ranged between 12.5-200 mg/ml for *S. aureus*, 6.25-200 mg/ml for *P. aeruginosa*, 50-200 mg/ml for *C. albicans*, 12.5-100 mg/ml for *M. audouinii* and 25 mg/ml for *M. furfur*. This implies that a higher concentration of antimicrobial agent is needed for killing the pathogen than for inhibiting their growth. The lowest MIC (3.12-25 mg/ml) and MBC/MFC (6.25-50 mg/ml) were seen in case of ethyl acetate seed extract against all the five tested pathogens. *P. aeruginosa* was expected the most sensitive strain to ethyl acetate extract among all the pathogens tested having lowest MIC i.e. 3.12 mg/ml followed by *S. aureus* (6.25 mg/ml), *M. audouinii* (6.25 mg/ml), *M. furfur* (12.25 mg/ml) and *C. albicans* (25 mg/ml).

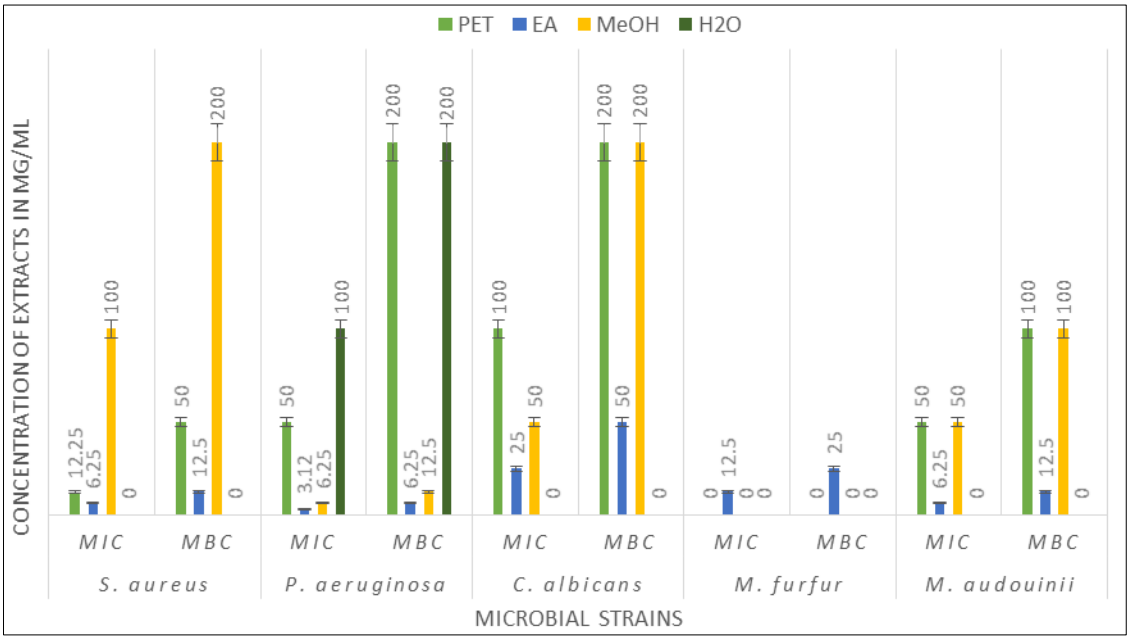

Fig 1: MIC, MBC/MFC of seed extracts of *H. laurifolia* against five tested pathogens

4. Discussion

The scalp is unique among skin areas as it possesses several unique features that aid in its critical role of protecting the head and heat conservation. Today in human society, looks of hair or hairstyle play an important role in the appearance and sexual signaling and changes in the appearance of hair and skin affect confidence and self-esteem in social life [20]. To improve the health of hair and scalp and to get rid of infections of scalp there are many allopathic drugs available in the market which may cause major side effects such as hair fall, alopecia etc. The latest trend shows that the herb based antimicrobials have an enormous therapeutic potential since they have no toxic effects on human beings. The present investigation revealed that the seed extracts of *H. laurifolia* possess promising antimicrobial efficacy against bacterial and fungal pathogens which may cause scalp infections. Different extracts contain varying concentrations of different phytochemical compounds. The variation in antimicrobial activity of different solvent extracts is due to the variation in bioactive constituents of the plants. Though, methanol and petroleum ether extracts also showed recognizable antimicrobial efficacy but, ethyl acetate seed extract exhibited best antimicrobial potency against all the five tested pathogens, in this context, this might be due to the effect of bioactive principles present in ethyl acetate seed extract such as flavonoids, steroids, tannins, glycosides and triterpenoids etc. [21]. The zone of inhibitions by ethyl acetate extract against both the tested bacteria were significantly greater than all the three tested concentrations of reference antibiotics. On the other hand, all the three fungal strains were found resistant to the tested concentration of griseofulvin but sensitive to different plant extracts especially ethyl acetate (Table 2). However, results of MIC and MBC revealed that, petroleum ether and methanol extracts also inhibit the growth of pathogens but ethyl acetate extract resist all the five tested pathogens through the minimal concentrations of the extract (Fig 1). This may indicate that the ethyl acetate extract contain some more potent bioactive compounds than other three extracts which could inhibit or kill the pathogenic microbiota of the scalp. Earlier studies demonstrated that methanol, ethanol, acetone and aqueous seed extracts of *H. wightiana* exhibited potent antimicrobial activity against *E. coli*, *Salmonella typhi*, *Proteus mirabilis* and *Klebsiella pneumoniae* [22]. Many pathogenic microorganisms such as *S. aureus*, *Escherichia coli* and *P. aeruginosa* etc. are resistant to many antimicrobial drugs [23]. However, seed extracts of *H. laurifolia* showed moderate to highest antimicrobial efficacy probably due to the availability of bioactive compounds in

high concentration. In particular, *H. laurifolia* contains flavonoids, steroids, tannins, glycosides and triterpenoids which are effective against bacterial and fungal strains. This shows the effectiveness of this plant against various pathogenic microorganisms.

5. Conclusion

This study gives the scientific credence that the seeds of *H. laurifolia*, which are traditionally utilized as a remedy of leprosy, have also potential as an antimicrobial agent for the treatment of scalp infections. Ethyl acetate seed extract exhibited best antimicrobial potency against all the tested strains followed by methanol, petroleum ether, and water extracts. This investigation concludes that the ethyl acetate seed extract of *H. laurifolia* is particularly promising in the context of scalp infections as it resists all the five tested pathogenic strains and exhibits effective zone of inhibitions which are recognizably greater than some tested standard antimicrobial drugs.

6. Acknowledgment

Current research work was supported by the grant from University Grants Commission (UGC), New Delhi, India. Authors are gratefully acknowledged to the Department of Botany and Microbiology, Gurukul Kangri University, Haridwar, Uttarakhand for providing facilities to enable this research to be carried out.

7. References

1. Shuster S. The aetiology of dandruff and the mode of action of therapeutic agents. Br J Dermatol. 1984; 111:235-242.
2. Jang SJ, Lim SH, Ko JH, Oh BH, Kim SM, Song YC *et al*. The investigation on the distribution of *Malassezia* yeasts on the normal Korean skin by 26S rDNA PCR-RFLP. Ann Dermatol. 2009; 21:18-26.
3. Sadia S, Tariq A, Shaheen S, Malik K, Khan F, Ahmad M *et al*. Ethnopharmacological profile of antiarthritic plants of Asia-A systematic review. Journal of Herbal Medicine. 2018. Doi: https://doi.org/10.1016/j.hermed.2018.08.003
4. Barrett JP, Vardulaki KA, Conlon C, Cooke J, Daza-Ramirez P, Evans EG *et al*. A systematic review of the antifungal effectiveness and tolerability of amphotericin B formulations. Clin Ther. 2003; 25(5):1295-1320.
5. Pandey MK, Singh MK, Singh RB. Mycotoxic potential of some higher plants. Plant Dis Res. 2002; 17:51-56.
6. Falagas ME, Bliziotis IA. Pandrug-resistant gram-negative bacteria: the dawn of the post-antibiotic era. Int J Antimicrob Agents. 2007; 29:630-6.
7. Barbosa LN, Rall VL, Fernandes AA, Ushimaru PI, da Silva PI, Fernandes AJ. Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathog Dis. 2009; 6:725-728.
8. Hazni H, Ahmad N, Hitotsuyanagi Y, Takeya K, Choo CY. Phytochemical constituents from *Cassia alata* with inhibition against methicillin-resistant *Staphylococcus aureus* (MRSA). Planta Med. 2008; 74:1802-1805.
9. Kumar MS, Kirubanandan S, Sripriya R, Sehgal PK. Triphala promotes healing of infected full-thickness dermal wound. J Surg Res. 2008; 144:94-101.
10. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. Journal of Ethnopharmacology. 2006; 103:25-35.
11. Jain D, Jain Y. Hair loss & herbal medicines. Global J Trad Med Sys. 2012; 1(1):13-15.
12. Jadav HR, Ruknuddin G, Harisha CR, Kumar PP. Preliminary Pharmacognostical profile of Tuvaraka (*Hydnocarpus laurifolia* (Dennst) Sleummer) seeds. Med J DY Patil Univ. 2016; 9:219-23.
13. Lucas DS. Study of Dravya-Materia Medica, Dravyaguna-Vijnana, Edn 1, Chaukhamba Visvabharati, Oriental, India, 2008.
14. Mathai BM, Joseph MM, Maniganda S, Nair JB, Arya JS, Karunakaran V *et al*. Guanidinium rich dendron-appended hydnocarpin exhibits superior anti-neoplastic effects through caspase mediated apoptosis. RSC Advances. 2016; 6:52772-52780.
15. Ahmed I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol. 1998; 62:183-193.
16. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008; 3:163-75. Available from: http://www.nature.com/doifinder/10.1038/nprot.2007.52.
17. Tura GT, Eshete WB, Tucho GT. Antibacterial efficacy of local plants and their contribution to public health in rural Ethiopia. 2017; 6(76):1-7. DOI 10.1186/s13756-017-0236-6
18. Ginovyan M, Petrosyan M, Trchounian A. Antimicrobial activity of some plant materials used in Armenian traditional medicine. BMC Complementary and Alternative Medicine. 2017; 17(50):1-9. DOI 10.1186/s12906-017-1573-y
19. Silva KVS, Lima MIO, Cardoso GN, Santos AS, Silva GS, Pereira FO. Inibitory effects of linalool on fungal pathogenicity of clinical isolates of *Microsporum canis* and *Microsporum gypseum.* Mycoses. 2017; 1-7. DOI: 10.1111/myc.12606
20. Grimalt RA. Practical guide to scalp disorders. J Investig Dermatol Symp Proc. 2007; 12:10-14.
21. Rao PS, Mohan GK. Protective profile of *Hydnocarpus laurifolia* on streptozotocin induced oxidative stress in rats. International Journal of Pharmaceutical Sciences and Research. 2017; 8(1):231-235.
22. Samuel S, Senthilkumar PK, Muthukkaruppan SM. Screening of antimicrobial activity of indian medicinal plants. Journal of Experimental Sciences. 2010; 1(6):25-31.
23. Aielloa AE, Cimiotti J, Della-Lattac P, Larson EL. A comparison of the bacteria found on the hands of ‘homemakers’ and neonatal intensive care unit nurses. J Hosp Infect. 2003; 54(4):310-315.