EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 3
417-422
Year: 2019

ANTIFUNGAL AND PRELIMINARY PHYTOCHEMICAL SCREENING OF FRUIT EXTRACTS OF *LYCIUM BARBARUM* LINN

Vinnashini S.[1], Anita Gnana Kumari A. V.*[1] and Anbu Jeba Sunilson J.[2]

[1]School of Pharmacy, KPJ Healthcare University College, Kota Seriemas, Nilai 71800, Negeri Sembilan, Malaysia.
[2]Department of Siddha Medicine, Faculty of Science, Tamil University, Thanjavur 613010 Tamil Nadu, India.

***Corresponding Author: Dr. Anita Gnana Kumari A. V.**
School of Pharmacy, KPJ Healthcare University College, Kota Seriemas, Nilai 71800, Negeri Sembilan, Malaysia.

Article Received on 10/01/2019 **Article Revised on 30/01/2019** **Article Accepted on 20/02/2019**

ABSTRACT
Opportunistic fungal infections have been a common element of morbidity and mortality within the immunosuppressed individuals while dermatophytosis is the superficial fungal infection caused by dermatophytic fungi with high affinity for keratinized tissue. The remedy of these infections is a remarkable challenge due to the resistance of antifungal drugs. The fruit of *Lycium barbarum* has been traditionally used in Chinese herbal medicine and they are considered as functional foods with wide range of pharmacological activities. The aim of this study was to evaluate the effect of *Lycium barbarum* (goji berry) fruit extract against fungal pathogens. In the present study aqueous and methanol extracts of fruit extract of *Lycium barbarum* were tested in vitro for their antifungal activity against *Candida albicans, Aspergillus niger* and *Trichophyton rubrum.* Methanol extract was the most potent with maximum zone of inhibition(24 & 25mm)against *Candida albicans* and *Trichophyton rubrum* and compared with standard drug fluconozole. The MIC and MBC values of the methanolic extract of *Lycium barbarum* fruit extract against *Candida albicans* and *Trichophyton rubrum* were found at the concentration of 125 and 250mg/ml. The phytochemical screening of extracts revealed the presence of flavonoid, saponin, polyphenol and volatile oil. Findings from the study revealed that methanol extract of *Lycium barbarum* shows significant antifungal activity against *Candida albicans* and *Trichophyton rubrum*. Further studies are required to understand the efficacy and the mechanism of action involved in the antifungal activity of the plant extract of *L barbarum.*

KEYWORDS: *Lycium barbarum fruits*, Ethanolic extracts, Aqueous extracts, Fungal pathogens.

INTRODUCTION
Fungal infection reports for significant rates of morbidity and mortality however, there is insufficient of systematic data on the incidence and popularity of human mycoses in Malaysia.[1] These infections have been generated serious problems worldwide in immune-suppressed patients. Worldwide, the infection and mortality rate are increasing due to opportunistic mycoses such as *Candida albicans, Aspergillus niger,* and *Trichiphyton rubrum* among patients with weak immunity.[2]

Candida infections generally arise in warm moist frame regions, such as underarms, mouth and diaper areas. Vaginal Candidiasis is a yeast infection, which is the most common form of vaginitis.[3] *Aspergillus niger* is known to be a filamentous ascomycete fungus which is universal in the environment and has been involved in the opportunistic infections of people. Opportunistic Infections is an infection caused by bacterial, viral, fungal, or protozoan pathogens that take advantage of a host with a weakened immune system.[4] *Trichophyton rubrum* is known as dermatophyte which cause superficial fungal infections. The dermatophytes are a subgroup of organism that have the capacity to penetrate-keratinized tissues, such as hair, skin and nails.[5]

The consequence of antifungal drugs to the use of modern medicine has elevated dramatically, over the past 30 years.[6] The azoles are known to be used as broadly under antifungal drugs. Although there are many azoles available in the market, yet fluconazole, posaconazole and voriconazole are most frequently used invasive fungal infections treatment. Azoles inhibit the biosynthesis of ergosterol and they are known to be fungistatic.[7] Fluconazole has wide activity against clinically compatible yeast including *Candida spp, Aspergillus niger* and *Trichophyton rubrum.*

Goji berry (*Lycium barbarum*) is primarily cultivated in China.[8] It has a bright, red coloured, ellipsoid and 1-2 cm long grape-like fruit. Goji berry has the highest nutritional value in the world and is also known to be a powerful antioxidant.[9] Traditionally, *L.barbarum* has been used many properties such as vision and immune

system-improving consequences, and assist of kidney and liver function, and as a remedy for respiratory related diseases.[10]

Studies indicates the effects of *Lycium barbarum* on aging, neuroprotection, general well-being, antidiabetics, antiglaucoma, anti-oxidant, immunomodulation, anti-tumor and anticytoprotection but only few studies proven on antifungal properties. This study is intended to determine the effect of *Lycium barbarum* fruit extract in treating fungal infection. Extract of *Lycium barbarum* exhibit antifungal activity against fungal pathogens due to the present of phytoconstituent such as solavetinone and cyperone which is a powerful antifungal compound as stated in the previous studies.[11]

MATERIALS AND METHODS

Collection, authentication and preparation of the extract

The *Lycium barbarum* which was available in dried form were bought from Ah Seong Chinese Traditional Shop from Klang Selangor. The plant materials were authenticated by Dr.Mohd Firdaus Ismail from University Putra Malaysia (Ref No: UPM/IBS/UB/H79/17). 600g of air dried powdered materials were separately soaked in 90% methanol and distilled water for 7 days. After 7 days the both extract were filtered using Whatmann No 1 sterile filter paper. The excess solvent in the methanolic extract was evaporated to dryness by using rotatory vacuum evaporator (Heidolph 300 LabroRota, Germany) at 125rpm, while the aqueous extract was dried by using heating mantle at 50°C. Both filtrates were transferred into sterile airtight container and placed on refrigerator until tested.[12] The extracted samples were kept in refrigerator at 4°C prior to use.

Test Organism

The extracts were tested on few common fungal species such as *Candida albicans*, *Aspergillus niger* and *Trichophyton rubrum*. The fungal strains were obtained from Pusat Perubatan Universiti Kebangsan Malaysia. The procured sample were sub-cultured and maintained in Sabouraud Dextrose Agar plate at 4°C.[13]

Culture Medium

The Muller Hilton agar medium was used for the antifungal activity and it was prepared by using distilled water, the pH of the media was maintained at 7.4. The media was sterilized in autoclave for 15 minutes at 121°C.

Standard Drug

Fluconazole (150mg) have been used as standard antibiotic for fungal infections. It disrupts fungal cell membrane by inhibiting the synthesis of ergosterol.[14]

Preliminary Qualitative Phytochemical

The various extracts of *Lycium barbarum* obtained were subjected to qualitative analysis to detect the presence of various phytochemical constituents such as alkaloids, carbohydrates, glycosides, proteins and amino acids, fats, flavonoids, steriods, coumarins, terpenoids, tannins, phytosterols, saponins, poly phenols and oils/resins by standard methods.

Preparation of *Lycium barbarum* Fruit Extract

The fruit extract of *Lycium barbarum* were prepared in concentration of 500mg/ml of distilled water.

Invitro Anti-Fungal Activity of *Lycium barbarum* Fruit Extracts by using Agar Well Diffusion Technique

The agar well diffusion technique is widely used to evaluate the antifungal activity of plant extracts of *Lycium barbarum*. The agar plate surface has been inoculated by spreading a volume of the *Candida albicans*, *Aspergillus niger*, and *Trichophyton rubrum* by using a cotton swab. Then in each plate, well or cup with a diameter of 6 to 8 mm is aseptically made with a sterile cork borer. Then, 0.1ml of standard drug (150mg/ml) aqueous and methanol fruit extract (500mg /ml) was introduced to each well on the medium. Then, agar plates were incubated under 25-30^{o}c for 72-96 hours. The antifungal agent diffuses in the agar medium and inhibits the development of the fungal strain tested.[15] Growth inhibition was quantified as inhibition circular zones, and their diameters were measured. All the plates were performed in triplicates.[16]

Determination of Minimum Inhibitory Concentration (MIC) of *Lycium barbarum* extracts against *Candida albicans, Aspergillus niger, Trichophyton rubrum*

The MIC was determined by the dilution method in which various concentration (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml was used. In a sterilised test tube, 2 ml of the Mueller Hinton broth, 1ml of the extract (500mg/mL) was added to the test tube, mixed well an serially diluted in other test tube containing 2 ml Mueller Hinton broth. Then 0.1ml of the fungal strain inoculum were added into the test tubes. DMSO was used as negative controls.[16] The tubes were kept for incubation at 22-27°C for 72-96 hours. After incubation, the minimum inhibitory concentration of extract was determined as based on the turbidity and absorbance values were measured at 630nm using UV-spectrophotometer. All samples were repeated in triplicate.[17]

Determination of Minimum Fungicidal Concentration of *Lycium barbarum* against *Candida albicans, Aspergillus niger* and *Trichophyton rubrum*

To determine minimum fungicidal concentration of *Lycium barbarum* fruit extracts, the lowest concentrations which inhibited fungal growth were inoculated on SDA plates and incubated at 25 °C for 72-96 hours. The lowest concentration of *Lycium barbarum* fruit extract that has no fungal growth was recorded as MFC.[18]

Statistical Analysis

The data was expressed as mean ± S.E.M. The assessment for MIC study was performed in triplicate and the data was subjected to one way analysis of variance (ANOVA) using Dunnett 'T' test and p values < 0.05 was considered as significant.

RESULTS

The colour, consistency and percentage of yield of fruit extract of *Lycium barbarum* were shown in the Table 1.

Table No 1: The nature and yield percentage.

No	Extract	Colour	Consistency	% Of Yield
1	Methanol	Dark Orange	Sticky Mass	57.84
2	Aqueous	Dark Brown	Sticky Mass	91.26

Qualitative phytochemical screening indicated that the aqueous and methanol fruit extract of *Lycium barbarum* contained active compounds as shown in Table 2. The aqueous extract showed the presence of alkaloid, protein, terpenoid, tannin, phytosterol, saponin, polyphenol, oil/resin and volatile oil. The methanol extract showed the presence of alkaloid, protein, flavonoid, terpenoid, tannin, phytosterol, polyphenol, oil/resin and volatile oil.

Table No 2: Phytoconstituent present in aqueous and methanol extract of *Lycium barbarum.*

Phytochemical Constituents	Aqueous Extract	Methanol Extract
Alkaloid	+	+
Carbohydrate	-	-
Protein	+	+
Fats	-	-
Flavonoid	-	+
Steroid	-	-
Terpenoid	+	+
Tannin	+	+
Phytosterol	+	+
Saponin	+	-
Polyphenol	+	+
Oil/Resin	+	+
Volatile oil	+	+

- Indicates absence of phytoconstituent
+ Indicates presence of phytoconstituent

The invitro antifungal activity of aqueous and methanol fruit extract of *Lycium barbarum* were summarized in Table 3, Figure 1, Figure 2 and Figure 3. The result showed that the aqueous extract have no activity against *Candida albicans, Aspergillus niger* and *Trichophyton rubrum.* The methanol extract are effective against *Candida albicans* and *Trichophyton rubrum* but no effective against *Aspergillus niger*. The methanol extract against *Trichophyton rubrum* showed higher activity than the standard drug.

Table No 3: Invitro antifungal activity of methanol and aqueous fruit extract of *Lycium barbarum* against *Candida albicans, Aspergillus niger* and *Trichophyton rubrum.*

Test Organism	Inhibition Zone (mm)		
	Standard Drug (Fluconazole)	Aqueous Extract	Methanol Extract
CANDIDA ALBICANS	26±0.88	13±5.67	24±0.88*
ASPERGILLUS NIGER	26±0.88	5±0.88	10±0.67
TRICHOPHYTON RUBRUM	17±1.45	17±0.88	25±0.58**

n= 3; ***p< 0.001 = high significant, **p< 0.01 = significant and *p< 0.05 = less significant

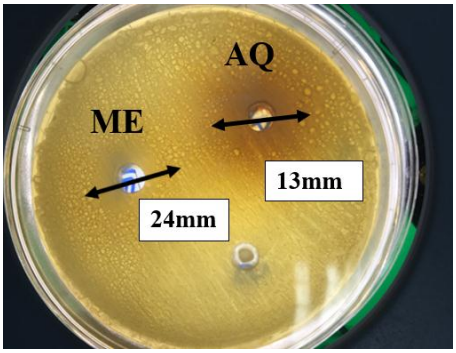


Figure 1: Zone of Inhibition in (mm) for antifungal activity of aqueous and methanol extract of *Lycium barbarum* against *Candida albicans.*

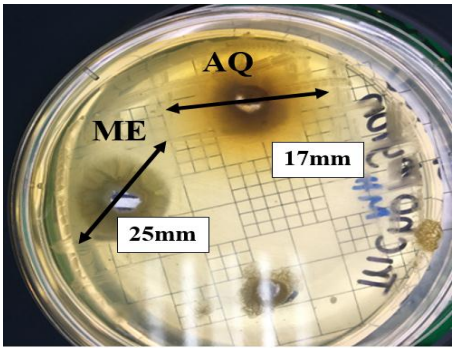
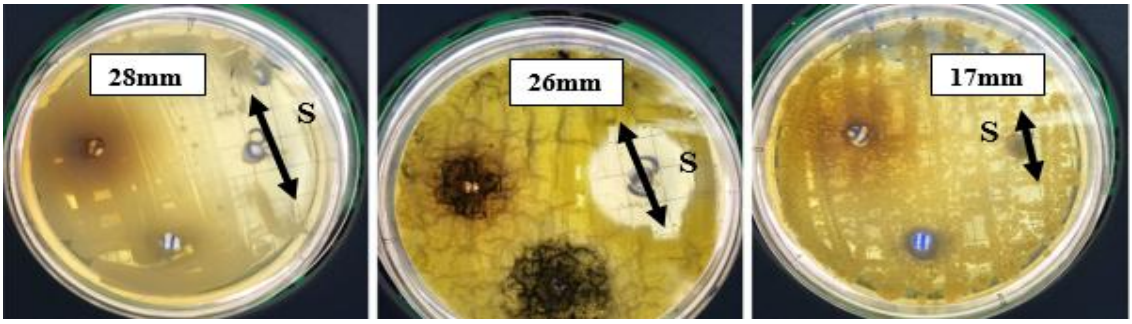


Figure 2: Zone of Inhibition in (mm) for antifungal activity of aqueous and methanol extract of *Lycium barbarum* against *Trichophyton rubrum.*



Candida albicans ***Aspergillus niger*** ***Trichophyton rubrum***

Figure 3: Zone of Inhibition in (mm) for antifungal activity of Fluconazole 150mg/mL against fungal strains.

The MIC values of methanol fruit extract of *Lycium barbarum* were determined by using dilution method. The methanol extract showed inhibitory effect against *Candida albicans* (0.67±0.02) and *Trichophyton rubrum (0.63±0.01)* at the concentation of 125mg/ml. The results were shown in Table 4.

Table No 4: Methanol fruit extract of *Lycium barbarum* against the *Candida albicans and Trichophyton rubrum* using Uv-Vis Spectrophotometer.

Test organism	Different concentration of methanol fruit extract of *Lycium barbarum* (mg/ml)					Standard drug Fluconazole (150mg/ml)
	500	250	125	62.5	31.25	
C.albicans	0.52±0.15	0.45±0.01	0.67±0.02**	1.28±0.02	1.68±0.06	0.38±0.01
T.rubrum	0.13±0.01	0.44±0.01	0.63±0.01**	1.25±0.01	1.35±0.02	0.32±0.02

n= 3; ***p< 0.001 = high significant, **p< 0.01 = significant and *p< 0.05 = less significant

The MFC values of methanol fruit extract of *Lycium barbarum* were determined by using plate method. The result were shown in Table 5. The methanol extract showed fungicidal effect on *Candida albicans* and *Trichophyton rubrum* at 250mg/ml.

Table No 5: Minimum Fungicidal Concentration (MFC) of *Lycium barbarum* against *Candida albicans,* and *Trichophyton rubrum.*

Test organism	Different concentration of methanol fruit extract of *Lycium barbarum* (mg/ml)		
	500	250	125
Candida albicans	-	-	+
Trichophyton rubrum	-	-	+

- Indicates no fungal growth
+ Indicates fungal growth

DISCUSSION

Invasive fungal infections, which include candidiasis, have improved in incidence international over the past two decades, and consequently, the use of antifungal drugs inclusive of azoles has extended. Antifungal drugs such as fluconazole and ketoconazole, have significant roles within the treatment of candidiasis and other invasive fungal infections but from time to time with

using these marketers, clinically essential toxic effects including skin rash, nausea, pores, increased liver enzyme (for fluconazole) gynecomastia, adrenal insufficiency and hepatotoxicity (for ketoconazole) are visible. Overtime, beneath a few medical settings, the efficacy of azoles has decreased due to accelerated resistance to the antifungals.

Table 1 shows the average percentage of dried sample of *Lycium barbarum* after the drying process. The table 1 shows the percentage of yield from the cold maceration extraction with different polarity of solvent. The aqueous extract gave the best percentage of yield for fruit extract of *Lycium barbarum* followed by methanol extract which is 91.26% and 57.84% respectively. The methanol extraction of *Lycium barbarum* had the lowest yield compare to aqueous extraction, but inversely the highest average growth inhibition.

These research were conducted on antifungal activity of *Lycium barbarum* against *Candida albicans, Aspergillus niger* and *Trichophyton rubrum*. The phytochemical qualitative analysis (Table 2) revealed that alkaloid, protein, terpenoid, tannin, phytosterol, saponin, polyphenol, oil/resin and volatile oil were present in the aqueous extracts of *Lycium barbarum* fruit extract. While in methanol extract revealed the presence of alkaloid, protein, flavonoid, terpenoid, tannin, phytosterol, polyphenol, oil/resin and volatile oil. In this study, the presence of flavonoid, saponin, polyphenol and volatile oil in the extract exhibit the antifungal activity.[19]

The agar diffusion assay have been used for evaluation of antifungal activity of these fruit extract of *Lycium barbarum*. The present study shows that the methanol fruit extract is most effective against fungal strains than the aqueous extract forms. The antifungal potential plants was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standards, Flucanazole (150mg/ml). The results revealed that all the methanol extracts are potent antifungal against *Candida albicans* and *Trichophyton rubrum.* Among the different solvents extracts studied methanol showed high degree of inhibition followed by aqueous extract.

In the antifungal activity, the methanol fruit extract of *Lycium barbarum* showed efficient antifungal activity against *Candida albicans* (24±0.88 mm) and *Trichophyton rubrum* (25±0.58 mm) and aqueous extracts were showed no antifungal activity against *Candida albicans* (13±5.67mm) and *Trichophyton rubrum* (17±0.88mm). The aqueous and methanolic extract for *Aspergillus niger* showed the no inhibition zone. From this study we can conclude that, the methanol extract of L*ycium barbarum* against *Candida albicans* are significant with p value of <0.05 while methanol extract for *Trichophyton rubrum* are high significant with p value of <0.01. *Tricophyton rubrum* is known as dermatophyte, which are responsible for the superficial fungal infection while *Candida albicans* are yeast that causes opportunistic fungal infection that effects the immune system. The fruit of *Lycium barbarum* are used traditionally to maintain healthy skin.[8] The presence of carotenoid in fruit of *Lycium barbarum* protect and help to maintain a healthy skin.[20] Therefore, the fruit of *Lycium barbarum* can used against *Trichophyton rubrum* species.

The minimum inhibitory concentration (MIC) is regarded as the lowest concentration that needed to inhibit the fungal growth.[21] In the present research, there is no growth for *Candida albicans* in methanol extract for the following concentration of 500mg/ml, 250mg/ml and 125mg/ml and it was identified by the absence of turbidity in Mueller Hilton broth. While no growth is observed at 500mg/ml, 250mg/ml, and 125mg/ml of methanol extract of *Trichophyton rubrum.* It was observed that the lower the extract concentration, the higher the visibility of fungal growth. The MIC should be done carefully as it can easily get affected by the environment, incubation condition and time of incubation.[22] The MIC value for methanol extract for both *Candida albicans* and *Trichophyton rubrum* is 125mg/ml. Thus, it revealed that the methanol extract of plant *Lycium barbarum* exhibit inhibitory activity which can be detected by the absence of turbidity in the test tubes. From this study we can conclude that, the methanol extract of *Lycium barbarum* against both *Candida albicans* (0.67±0.02) and *Trichophyton rubrum* (0.63±0.01) are highly significant with a p value of <0.01. This study also revealed that methanol extract of *Lycium barbarum* shows significant antifungal activity against *Candida albicans* and *Trichophyton rubrum*.

The different concentration of methanol extract were used from the MIC test have been experimented on the *Candida albicans* and *Trichophyton rubrum* to determine the MFC value. The result revealed that, 250mg/ml strength is needed to act as fungicidal for both the fungal strains. Besides that, the result shows that the methanol extract of the plant has remarkable minimum fungicidal concentration (MFC) activity. This may due to present of flavonoid in the methanol extract plant. Flavonoids are a compound that active against variety of microorganism. In the previous research paper by[23] stated on antifungal activity of flavonoids which serve as potential substitute to fungicides.

CONCLUSION

In conclusion, the methanol extracts of *Lycium barbarum* fruit extract exhibited good antifungal activities and were capable of reducing growth of *Candida albicans* and *Trichophyton rubrum*. The in-vitro assessment of the plant extracts against the test organism and the phytochemical compounds present in the plants shows good inhibitory activity of the fruit on fungi. It was found that the antifungal activity of methanol extract of

lycium barbarum is higher than the *Lycium barbarum* aqueous extract. The *Lycium barbarum* fruit methanol extract used in this study could be useful in the treatment of fungal infections.

ACKNOWLEDGEMENTS

The authors would like to thank the Management, KPJ Healthcare University College, Kota Seriemas, Nilai, Malaysia, for their continuous encouragement and support.

REFERENCES

1. Rukumani Devi V and David W: Estimation of serious fungal disease burden in Malaysia, 2016.
2. Yoon, Hee Jung, Choi, Hwa Young, Kim, Young Kwon, Song, Yeong Jun, and Ki, Moran: Prevalence of fungal infections using National Health Insurance data from 2009-2013, South Korea. Epidemiology and health, 2014; 36.
3. Nobile, Clarissa J and Alexander D: Candida albicans biofilms and human disease. Annual review of microbiology, 2015; 69: 71-92.
4. Baker and Scott E: Aspergillus niger genomics past, present and into the future. Medical mycology, 2006; 44: 17-21.
5. Blutfield, Matthew S, Lohre, Jenna M, Pawich, Derek A, Vlahovic and Tracey C: The immunologic response to Trichophyton rubrum in lower extremity fungal infections. Journal of Fungi, 2015; 1: 130-137.
6. Richardson MD. Changing patterns and trends in systemic fungal infections. Journal of Antimicrobial Chemotherapy, 2005; 1: 56: 5-11.
7. Meletiadis J, Antachopoulos C, Stergiopoulou T, Pournaras S, Roilides E, and Walsh TJ. Differential fungicidal activities of amphotericin B and voriconazole against Aspergillus species determined by microbroth methodology. Antimicrobial agents and chemotherapy, 2007; 1: 51: 3329-37.
8. Amagase H and Farnsworth NR. A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of Lycium barbarum fruit (Goji). Food research international, 2011; 1: 44: 1702-17.
9. Li Q, Yu X, and Gao JM. A novel method to determine total sugar of goji berry using FT-NIR spectroscopy with effective wavelength selection. International Journal of Food Properties, 2017; 18: 20: 478-88.
10. Jurado SR, Leão BC, de Oliveira SB, Rosa D and da Costa FS. Ingestion of Goji Berry (Lycium Barbarum) Evaluation on Plasma Levels of Total Cholesterol, Lipid Fractions, Glycaemia, Serotonin and Arterial Pressure. Cardiolog Res Cardiovasc Med, 2017.
11. Gogoasa I, Alda L, Rada M, Negrea P, Negrea A, Bordean DM, Velciov A, Draghici GA and Gergen I. Goji berries (Lycium barbarum) as a source of trace elements in human nutrition. Journal of Agroalimentary Processes and Technologies, 2014; 20: 369-72.
12. Al-Terehi M, Al-Saadi AH, Zaidan HK, Alkaim ZH, Habeeb RA and Majed N. Some herbal medicinal plants activity against Candida spp which resistance to antifungal drugs. International Journal of Pharm Tech Research, 2015; 8: 146-50.
13. Sharma KK, Kotoky J, Kalita JC, Barthakur R. Evaluation of antidermatophytic activity of Ranunculus sceleratus and Pongamia pinnata available in North Eastern Region of India. Asian Pacific Journal of Tropical Biomedicine, 2012; 1: 2: S808-11.
14. Kothavade RJ, Kura MM, Valand AG and Panthaki MH. Candida tropicalis: its prevalence, pathogenicity and increasing resistance to fluconazole. Journal of medical microbiology, 2010; 1: 59: 873-80.
15. Balouiri M, Sadiki M and Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis, 2016; 1: 6: 71-9.
16. Paz M, Gúllon P, Barroso MF, Carvalho AP, Domingues VF, Gomes AM, Becker H, Longhinotti E and Delerue-Matos C. Brazilian fruit pulps as functional foods and additives: Evaluation of bioactive compounds. Food Chemistry, 2015; 1: 172: 462-8.
17. Alghazeer R, Whida F, Abduelrhman E, Gammoudi F and Naili M. In vitro antibacterial activity of alkaloid extracts from green, red and brown macroalgae from western coast of Libya. African Journal of Biotechnology, 2013; 12: 7086-91.
18. Shukla R, Singh P, Prakash B and Dubey NK. Antifungal, aflatoxin inhibition and antioxidant activity of Callistemon lanceolatus (Sm.) Sweet essential oil and its major component 1, 8-cineole against fungal isolates from chickpea seeds. Food Control, 2012; 1: 25: 27-33.
19. Kulczyński B and Gramza-Michałowska A. Goji berry (Lycium barbarum): Composition and health effects–A review. Polish Journal of Food and Nutrition Sciences, 2016; 1: 66: 67-76.
20. Zhao H, Alexeev A, Chang E, Greenburg G and Bojanowski K. Lycium barbarum glycoconjugates: effect on human skin and cultured dermal fibroblasts. Phytomedicine, 2005; 10: 12: 131-7.
21. Sen A and Batra A. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: Melia azedarach L. Int J Curr Pharm Res., 2012; 4: 67-73.
22. Al-Haj NA, Mashan NI, Shamsudin MN, Mohamad H, Vairappan CS and Sekawi Z. Antibacterial activity in marine algae Eucheuma denticulatum against Staphylococcus aureus and Streptococcus pyogenes. Research Journal of Biological Sciences, 2009; 4: 519-24.
23. Lourenço RM, da Silva Melo P an de Almeida AB. Flavonoids as antifungal agents. InAntifungal Metabolites from Plants 2013 (pp. 283-300). Springer, Berlin, Heidelberg.