Original Article

Antifungal effects of tulsi, garlic, cinnamon and lemongrass in powder and oil form on *Candida albicans*: An *in vitro* study

Mitul Prajapati[1], Monali Shah[1], Amena Ranginwala[1], Prakhar Agrawal[1], Dhruval Acharya[2], Shreya Thakkar[2]

[1]Department of Oral and Maxillofacial Pathology, Ahmedabad Dental College and Hospital, [2]Oral Pathologist, Private Practitioner, Ahmedabad, Gujarat, India

Abstract

Introduction: The use of plants for treating diseases is as old as the human species. Medicinal plants have been a major source of therapeutic agents for alleviation and cure of diseases. **Objectives:** The objective of the study was to evaluate and compare the antifungal activity of garlic, cinnamon, lemongrass and tulsi in powder and oil form at different concentrations on *Candida albicans*.

Materials and Methods: Powder and oil of garlic, cinnamon, lemongrass and Tulsi dissolved in inert solvent dimethyl formamide to obtain different concentration. Stock solution of different concentration was inoculated on Petri plates containing *C. albicans* and incubated at 30°C for 48 h. The inhibition zones were measured in millimeters using Vernier caliper. The collected data were analyzed using statistical test like mean value and one-way analysis of variance.

Results: Maximum zone of inhibition for the *C. albicans* was 42 mm at concentrations of 50% for the oil of lemongrass; followed by cinnamon 40 mm, garlic 24 mm and tulsi 20 mm. The *P* value obtained 0.050, 0.040, 0.036 and 0.031 were found to be statically significant for *C. albicans* at 20%, 30%, 40% and 50% concentrations of the various oil preparations, respectively. The *P* value obtained 0.043, 0.033, 0.032 and 0.027 were found to be statically significant for *C. albicans* at 20%, 30%, 40% and 50% concentrations of various plant powder, respectively.

Conclusions: Lemongrass and cinnamon oil shows best antifungal effect against *C. albicans* as compared to garlic and tulsi. Compared to powder preparations, the oil preparations are better to inhibit the growth and higher the concentrations, greater the zone of inhibition seen in all the plant extracts and in oil.

Keywords: *Candida albicans*, medicinal plants, plant preparations

Address for correspondence: Dr. Mitul Prajapati, Ahmedabad Dental College and Hospital, Bhadaj Ranchhodpura Road, Nr. Science City, Kalol, Gandhinagar - 382 115, Gujarat, India.
E-mail: opadc08@gmail.com

Submitted: 18-Sep-2020, **Accepted:** 06-Jul-2021, **Published:** 31-Aug-2021

INTRODUCTION

Candida albicans represent the most permeative fungal pathogen colonizing humans. *C. albicans* exists in two forms – pseudohyphae and yeast forms – a trait known as dimorphism. The yeast form is believed to be innocuous, but the hyphae form is usually associated with invasion into the

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/0973-029X.325233

How to cite this article: Prajapati M, Shah M, Ranginwala A, Agrawal P, Acharya D, Thakkar S. Antifungal effects of tulsi, garlic, cinnamon, and lemongrass in powder and oil form on *Candida albicans*: An *in vitro* study. J Oral Maxillofac Pathol 2021;25:306-12.

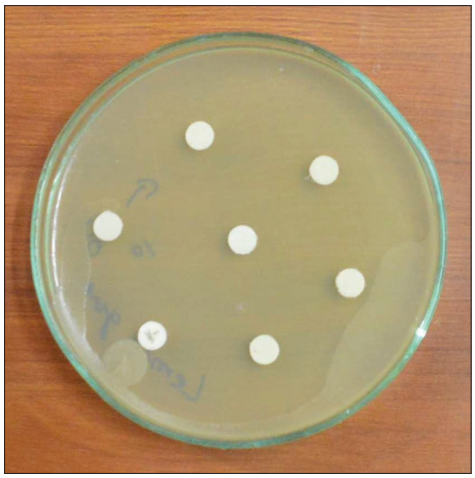

Figure 1: Inhibition zone at 50% concentration of lemongrass oil for Candida albicans

Figure 2: Inhibition zone at 50% concentration of lemongrass powder for Candida albicans

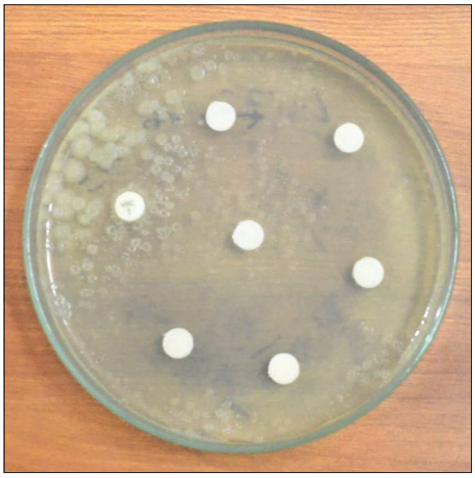

Figure 3: Inhibition zone at 50% concentration of cinnamon oil for Candida albicans

Figure 4: Inhibition zone at 50% concentration of cinnamon powder for Candida albicans

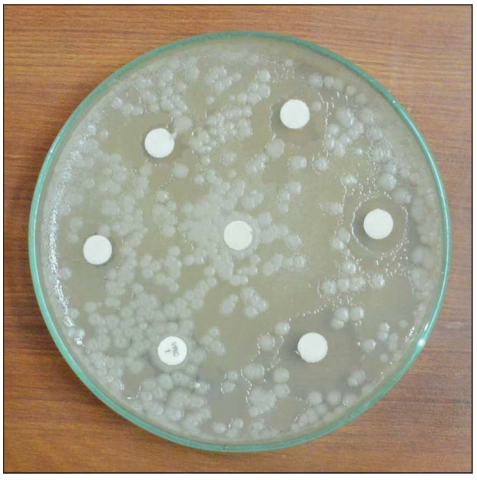

Figure 5: Inhibition zone at 50% concentration of garlic oil for Candida albicans

Figure 6: Inhibition zone at 50% concentration of garlic powder for Candida albicans

host tissue. This transition from a benign yeast type to highly invasive hyphae type depends on changes in the host defences.

Candidiasis is a most commonly observed opportunistic infection in oral cavity often referred to as thrush.[1] The

Figure 7: Inhibition zone at 50% concentration of tulsi oil for Candida albicans

Figure 8: Inhibition zone at 50% concentration of tulsi powder for Candida albicans

antimicrobial properties of various plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives. A few of them are cinnamon *(Cinnamomum zeylanicum)*, garlic (*Allium sativum*), tulsi (*Ocimum tenuiflorum*) and Lemongrass (*Cymbopogon*).

Garlic (*A. sativum*) is species in the onion genus, Allium. It has been used as both food and medicine in many cultures for thousands of years. Garlic has antiseptic, antimicrobial and antifungal properties. It is a good source of Vitamin B6, Vitamin C, manganese and phosphorus. The sulfur-containing compound alliin, ajoene, diallyl polysulfide's and Mailard reaction products are attributed to the therapeutic effects of garlic.[2] Cinnamon *(Cinnamum verum)* is a spice obtained from the inner bark of several trees from the genus Cinnamomum. It is principally employed in cookery as a condiment and flavoring material. It has a antioxidants, anti-inflammatory, antiviral, antibacterial, antifungal and antiallergic properties.[3] A tulsi (*Ocimum sanctum*) plant of Indian Basil occupies an important place in the Hindu religion. The name tulsi denotes "the incomparable one." Tulsi is used in many conditions such as fever, common cold, sore throat, and respiratory disorders. It has a germicidal, bactericidal, antiedematous, antimicrobial and anti-inflammatory properties.[4] Lemongrass (*Cymbopogon*) is plants in grass family. Lemongrass has hypnotic, anticonvulsant, antioxidant, antifungal, antibacterial, analgesic, antiemetic, antitussive and antiseptic effects. It helps in digestion, improves oral health and controls bad breadth.[5]

Hence, this study attempts to summarize the *in vitro* study of the antifungal properties of various plants such as cinnamon (*C. zeylanicum*), garlic (*A. sativum*), tulsi (*O. tenuiflorum*) and lemongrass (*Cymbopogon*) on *C. albicans*.

MATERIALS AND METHODS

The present study was design to evaluate and compare the antifungal activity of garlic, cinnamon, lemongrass and tulsi in powder and oil form at different concentrations on *C. albicans*.

Sample collection

Fresh garlic bulbs, cinnamon bark sticks and leaves of lemongrass and tulsi were collected from local market in Ahmedabad and 100% pure form of essential oils of same plants were purchased from Devinez company. Thirty-two Sabouraud dextrose agar media for *C. albicans* were taken.

Preparation of plant extracts (powder form)

Garlic bulbs, cinnamon bark sticks and leaves of lemongrass and tulsi were collected and cleaned twice using distilled water and ground to fine powder using a mechanical grinder. Twenty gram of each plant powder dissolved in 100 ml of distilled water in sterile mortar and pestle and then filtered using Whatman No. 1 filter paper and collected in a 250 ml glass flask. Flasks were then plugged with cotton and kept in refrigerator at 4°C for 24 h and then filtered and kept in a hot air oven for 5–7 days at 30°C ± 2°C to completely evaporate the solvent and to get a black shining crystal powder form. One gram of each extract was diluted with 10 ml of an inert solvent dimethyl formamide (DMFO) to obtain 10% concentration. This concentration was further diluted to obtain 20%, 30%, 40% and 50% concentrations and stored in sterile test tube.

Preparation of plant oils

Essential oils of tulsi, garlic, cinnamon and lemongrass were purchased from the local market in pure 100% form. One milliliter oil of all plant was dissolved in 10 ml of DMFO to obtain 10% concentration. This concentration

Table 1: Comparison of zone of inhibition (mm) for *Candida albicans* at different concentrations for various plants powder and oils

Plants	Zone of inhibition (mm) at different concentration											
	10%		20%		30%		40%		50%			
	Oil formulations	Powder formulations	Oil formulations	Powder formulations	Oil formulations	Powder formulations	Oil formulations	Powder formulations	Oil formulations	Powder formulations		
Cinnamon	16	13	24	16	30	24	35	28	40	32		
Garlic	12	12	14	12	18	16	21	18	24	21		
Lemongrass	24	21	27	23	31	26	36	30	42	34		
Tulsi	8	8	10	10	13	12	16	14	20	17		

Table 2: Statistical analysis of various plants powder at 10%, 20%, 30%, 40% and 50% on *Candida albicans*

Concentration (%)	*n*	Mean±SD
10	4	13.5000±5.44671
20	4	15.2500±5.73730
30	4	19.5000±6.60808
40	4	22.5000±7.72442
50	4	26.0000±8.28654
Total	20	19.3500±7.69330

SD: Standard deviation

Table 3: One-way ANOVA analysis for the mean comparison of various plant powder at 10%, 20%, 30%, 40% and 50% on *Candida albicans*

	Mean square	*F*	Significant
Between groups	105.200	2.242	0.113
Within groups	46.917		

Table 4: Statistical analysis of various plant oils at 10%, 20%, 30%, 40% and 50% concentration on *Candida albicans*

Concentration (%)	*n*	Mean±SD
10	4	15.0000±6.83130
20	4	18.7500±8.05709
30	4	23.0000±8.90693
40	4	27.0000±10.03328
50	4	31.5000±11.12055
Total	20	23.0500±10.07067

SD: Standard deviation

Table 5: One-way ANOVA analysis for the mean comparision of various plant oils at 10%, 20%, 30%, 40% and 50% on *Candida albicans*

	Mean square	*F*	Significant
Between groups	170.300	2.051	0.139
Within groups	83.050		

was further diluted to obtain 20%, 30%, 40% and 50% concentrations and stored in sterile test tube. The stock solution of positive control – voriconazole was also prepared by dissolving 100 mg in 10 ml of sterile distilled water to get the 10 mg/ml. Stock solutions of each plant extracts and oils were labeled.

Preparation of culture media

Freeze dried form of the microorganism *C. albicans was* obtained from Microbial Type Culture Collection, Chandigarh. The ampules containing freeze dried forms of microorganism were opened and content mixed with distilled water (0.4 ml). The obtained mixture was stirred using a sterile stirrer and kept standing for half an hour. Each prepared mixture then aseptically inoculated and evenly spread using sterile "L" rod or "Swab" on the surface of sterile culture media plate to obtain the primary Culture plates. For *C. albicans*, the culture media plate sabouraud dextrose agar (90 mm) was incubated for 30°C for 48 h and used for secondary culture procedure. Blank discs were

Table 6: Statistical comparison (one-way ANOVA) of various plant powder at different concentration on *Candida albicans*

Concentration (%)	Plant extracts	Inhibition zone (mm)	SD	*P*
10	Cinnamon	13	0.042	0.054
	Garlic	12	0.045	
	Lemongrass	21	0.026	
	Tulsi	8	0.068	
20	Cinnamon	16	0.072	0.043
	Garlic	12	0.096	
	Lemongrass	23	0.050	
	Tulsi	10	0.115	
30	Cinnamon	24	0.083	0.033
	Garlic	16	0.124	
	Lemongrass	26	0.076	
	Tulsi	12	0.165	
40	Cinnamon	28	0.110	0.032
	Garlic	18	0.172	
	Lemongrass	30	0.103	
	Tulsi	14	0.221	
50	Cinnamon	32	0.129	0.027
	Garlic	21	0.197	
	Lemongrass	34	0.122	
	Tulsi	17	0.244	

SD: Standard deviation

Table 7: Statistical comparison (one-way ANOVA) of various plant oil preparation at different concentration on *Candida albicans*

Concentration (%)	Plant extracts	Inhibition zone (mm)	SD	*P*
10	Cinnamon	16	0.043	0.064
	Garlic	12	0.057	
	Lemongrass	24	0.028	
	Tulsi	8	0.085	
20	Cinnamon	24	0.067	0.050
	Garlic	14	0.115	
	Lemongrass	27	0.060	
	Tulsi	10	0.161	
30	Cinnamon	30	0.089	0.040
	Garlic	18	0.148	
	Lemongrass	31	0.086	
	Tulsi	13	0.206	
40	Cinnamon	35	0.115	0.036
	Garlic	21	0.191	
	Lemongrass	36	0.111	
	Tulsi	16	0.251	
50	Cinnamon	40	0.139	0.031
	Garlic	24	0.232	
	Lemongrass	42	0.132	
	Tulsi	20	0.278	

SD: Standard deviation

impregnated. Required concentration of the various plant extracts and oils such as 10%, 20%, 30%, 40%, and 50% applied immediately on the surface of inoculated plates. A comparison antibiotic (positive control) test was made using commercial disk of voriconazole for *C. albicans*. Negative control of inert solvent DMFO was introduced into same plate. The fungal strains were incubated at 30°C for 48 h. The plates were duplicated for each concentration. Finally, the inhibition zones(diameter of translucent zones, from the center of each disk) were measured in millimeters using Vernier caliper [Figures 1-8].

Statistical analysis

The collected data were analyzed using statistical tests such as mean value and one-way analysis of variance.

RESULTS

The present study was carried out to evaluate the antifungal activity of plants such as cinnamon (*C. Zeylanicum*), garlic (*A. sativum*), tulsi (*O. tenuiflorum*) and lemongrass (*Cymbopogon*) in powder and oil form at various concentrations (10%, 20%, 30%, 40% and 50%) against *C. albicans*. In the present study, positive control for *C. albicans* – Voriconazole showed the zone of inhibition of 36 mm for all the concentrations. The negative control showed no zone of inhibition at all. The present study showed that the maximum zone of inhibition for the C. albicans was 42 mm at concentrations of 50% for the oil of lemongrass, followed by cinnamon 40 mm, garlic 24 mm and tulsi 20 mm [Table 1]. The collected data were analyzed using statistical test such as for powder preparations mean value [Table 2], One-way ANOVA [Table 3] and for oil preparations mean value [Table 4], One-way ANOVA [Table 5]. The P value obtained 0.043, 0.033, 0.032 and 0.027 were found to be statically significant for C. albicans at 20%, 30%, 40% and 50% concentrations of various plant extracts (powder), respectively. The P value obtained 0.054 was found to be statistically nonsignificant for the C. albicans at 10% concentration of the various plant extracts [Table 6]. The *P* value obtained 0.050, 0.040, 0.036 and 0.031 were found to be statically significant for *C. albicans* at 20%, 30%, 40% and 50% concentrations of the various oil preparations, respectively. The *P* value obtained 0.064 was found to be statistically nonsignificant for the *C. albicans* at 10% concentration of the oil preparations [Table 7]. Compared to powder preparations of various plants, the oil preparations are better to inhibit the growth at different concentrations and also the higher the concentrations, greater the zone of inhibition seen in all the plant extracts and in oil.

DISCUSSION

Candidiasis is a common opportunistic fungal infection of oral cavity, which is most commonly caused by the fungus *C. albicans*. It is most commonly seen in patients with an impaired immune system. All commercially available antifungal drugs with prolonged use may have negative effect on human health, and hence an alternative therapy with minimal side effects is desirable. Hence, the search for the alternative product continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals. Ayurveda is the traditional nature

healing system of India and is quickly gaining popularity. The present study was undertaken to assess the role of various plant extracts and essential oils such as tulsi *(O. sanctum),* cinnamon *(C. verum),* garlic *(allium sativum), and* Lemongrass *(Cymbopogon)* against *C. albicans*. Our study showed that lemongrass oil and powder both have an antifungal activity on *C. albicans*. The inhibitory zone increases with the increase in concentration of lemongrass oil and powder. These findings are in accordance with Pokpong Amornvit *et al*.,[6] who demonstrated antifungal activity at the concentration of 0.20% or at higher level of lemongrass oil by well-diffusion method. These findings are also similar to Tyagi and Malik,[7] Abe *et al*.,[8] Basera *et al.*[9] (2019), and Madeira *et al*.[10] found inhibition zone of 12 mm, 11 mm, 10 mm, 9 mm, and 7.5 mm at concentration of 0.63 mg/ml, 0.31 mg/ml, 0.16 mg/ml, 0.08 mg/ml and 0.04 mg/ml, respectively, which is similar to our findings that zone of inhibition increases with the increase in the concentration of lemongrass powder. Madeira *et al*.[10] also found antifungal activity on *C. albicans* is increased with increase in the concentration of lemongrass powder. Our study showed that cinnamon oil and powder both have an antifungal activity on *C. albicans*. Fani and Kohanteb[11] found inhibition zone of 8 mm, 13 mm, 27 mm and 54 mm at concentration of cinnamon oil at 12.5%, 25%, 50% and 100%, respectively, on *C. albicans* which is similar to our findings. Mahmood[12] found antifungal activity of cinnamon powder with higher concentration which is similar to our study. Allicin, an active compound in garlic, has antifungal activity. It downregulates the putative virulence gene, SIR2 in *C. albicans*.[13] Our study showed that garlic oil and powder both have an antifungal activity on *C. albicans*. These findings are in accordance with, Lemar[14] (2005) who found antifungal activity of Garlic oil at 50% concentration which shows 25 mm zone of inhibition and antifungal activity of garlic powder at 50% concentration which shows 20 mm zone of inhibition. Shuford *et al*.[15] and Shuford[15] (2005) found similar zone of inhibition activity on *C. albicans* with using higher concentration of garlic powder. Our study showed that tulsi oil and powder both have an antifungal activity on *C. albicans*. Devkatte *et al*.[16] found inhibition zone of 12 mm at 25% concentration of tulsi oil on *C. albicans* which is similar to our findings. Arora *et al*.[17] found inhibitory activity of tulsi powder on *C. albicans* at higher concentration. Pathak[18] found inhibitory zone increase with the increase in concentration of tulsi powder on *C. albicans* which is similar to our study. Subramaniam *et al*.[19] found inhibitory zone of 9.10 mm, 11.13 mm and 13.45 mm at 30%, 60% and 90% concentration of tulsi powder.

The addition of antimicrobial and antifungal agents to dentifrices, mouthwashes and varnishes increases the effect of mechanical oral hygiene procedure. When this antimicrobial and antifungal agent derived from plant, the undesirable effects of synthetic drugs can be overcome. Moreover, these phytochemicals produce other biological activities such as induction of immunity, which indirectly reduces the risk of oral diseases. Recently, some plants have also been shown to potentiate the activity of antimicrobial and antifungal agents against resistant strains, introducing the concept of resistance modification.

CONCLUSIONS

The present study showed that, as compared to powder preparations, oil preparations are better to inhibit the growth. Higher the concentrations greater the zone of inhibition is seen in all the plant powder and in oil. Lemongrass and Cinnamon oil shows best antifungal effect against *C. albicans* as compared to garlic and tulsi. Our study suggests that the inclusion of natural products with antimicrobial and antifungal properties in routine diet may helpful in combating and preventing various infectious diseases. However, further studies with multidisciplinary approach on a larger scale and with clinical trials will aid in giving clear evidence to confirm the antimicrobial and antifungal action and general safety.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Bommanavar SB, Gugwad S, Malik N. Phenotypic switch: The enigmatic white-gray-opaque transition system of *Candida albicans*. J Oral Maxillofac Pathol 2017;21:82-6.
2. Bayan L, Koulivand PH, Gorji A. Garlic: A review of potential therapeutic effects. Avicenna J Phytomed 2014;4:1-14.
3. Rao PV, Gan SH. Cinnamon: A multifaceted medicinal plant. Evid Based Complement Alternat Med 2014;2014:642942.
4. Cohen MM. Tulsi-*Ocimum sanctum*: A herb for all reasons. J Ayurveda Integr Med 2014;5:251-9.
5. Shah G, Shri R, Panchal V, Sharma N, Singh B, Mann AS. Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (Lemon grass). J Adv Pharm Technol Res 2011;2:3-8.
6. Amornvit P, Choonharuangdej S, Srithavaj T. Lemongrass-incorporated tissue conditioner against *Candida albicans* culture. J Clin Diagn Res 2014;8:ZC50-2.
7. Tyagi AK, Malik A. Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*: Microscopic observations and chemical characterization of *Cymbopogon citratus*. BMC Complement Altern Med 2010;10:65.
8. Abe S, Sato Y, Inoue S, Ishibashi H, Maruyama N, Takizawa T, *et al*. Anti-*Candida albicans* activity of essential oils including

Lemongrass (*Cymbopogon citratus*) oil and its component, citral. Nihon Ishinkin Gakkai Zasshi 2003;44:285-91.
9. Basera P, Lavania M, Agnihotri A, Lal B. Analytical investigation of *Cymbopogon citratus* and exploiting the potential of developed silver nanoparticle against the dominating species of pathogenic bacteria. Front Microbiol 2019;10:282.
10. Madeira PL, Carvalho LT, Paschoal MA, de Sousa EM, Moffa EB, da Silva MA, *et al. In vitro* effects of lemongrass extract on *Candida albicans* biofilms, human cells viability, and denture surface. Front Cell Infect Microbiol 2016;6:71.
11. Fani MM, Kohanteb J. Inhibitory activity of *Cinnamon zeylanicum* and *Eucalyptus globulus* Oils on *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida* species isolated from patients with oral infections. J Dent 2011;11:14-22.
12. Mahmood SN. Antifungal activity of *Cinnamon zeylanicum* and *Eucalyptus microtheca* crude extracts against food spoilage fungi. Euphrates J Agric Sci 2012;4:26-39.
13. Said MM, Watson C, Grando D. Garlic alters the expression of putative virulence factor genes SIR2 and ECE1 in vulvovaginal *C. albicans* isolates. Sci Rep 2020;10:3615.
14. Lemar KM, Passa O, Aon MA, Cortassa S, Müller CT, Plummer S, *et al.* Allyl alcohol and garlic (*Allium sativum*) extract produce oxidative stress in *Candida albicans*. Microbiology (Reading) 2005;151:3257-65.
15. Shuford JA, Steckelberg JM, Patel R. Effects of fresh garlic extract on *Candida albicans* biofilms. Antimicrob Agents Chemother 2005;49:473.
16. Devkatte AN, Zore GB, Karuppayil SM. Potential of plant oils as inhibitors of *Candida albicans* growth. FEMS Yeast Res 2005;5:867-73.
17. Arora T, Kang RS, Mann JS, Khurana NS, Aggarwal R, Walia G. Antimicrobial activity of herbal extracts against recalcitrant endodontic pathogens: An original *in vitro* study. Saint Int Dent J 2015;1:28-32.
18. Pathak AK. Anti-candida activity of aqueous extracts of some herbals. Indian J Fund Appl Life Sci 2012;2:1-6.
19. Subramaniam G, Tewari BB, Comathinayagam R. Studies of antimicrobial properties of different leaf extracts of Tulsi against human pathogens. Am Int J Contemp Res 2014; 4:149-157.