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Phytochemical analysis and synergetic effects on antifungal properties of garlic and ginger extracts along with honey and lemon against *Candida* spp.

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The present study was undertaken to investigate antifungal activity of garlic and ginger extracts and its synergetic effects against *Candida* spp. Antifungal activities of these extracts were tested *in-vitro* using well diffusion method. The extracts i.e., 100, 75 and 50 % showed significant antifungal activity against *Candida* spp. The less activity was observed in the low concentration of 25%. Garlic and ginger extracts along with honey and lemon were tested for antifungal activity in different combinations. Synergetic effects were observed when ginger and garlic extract was used along with other components. However, minimum inhibitory concentrations of different combinations along with ginger and garlic had a significant antifungal activity on *Candida albicans*. However, there was no significant activity when the extracts were used individually, especially on C. *krusei* and *C. tropicalis* strains. The phytochemical analysis revealed that ginger and garlic are rich in alkaloids, saponins, cardiac glycosides, steroids, flavanoiods, carotenoids, phenolic components, terpenoids, antraquinone, oxalates and phytates. The garlic extracts were devoid of tannins. It was concluded that there is significant activity in inhibiting the growth of yeast, *Candida* spp. the opportunistic pathogens. Therefore, ginger extracts might have promise in the treatment of *Candida* infections, and further *in-vitro* research is needed and *in-vivo* to obtain better results.

Keywords: *Allium sativum, Candida,* honey, lemon, *Zingiber officinale.*

INTRODUCTION

The therapeutic effects of plants and natural products are well established in contemporary medical science. Ginger (*Zingiber officinale*) is easily available universally acceptable and relatively inexpensive and well tolerated spice used by most of the populations. The plant *Z. officinale* belongs to Zingiberaceae family and the zingiberous plants have strong aromatic and medicinal properties (Supreetha et al., 2011). Garlic (*Allium sativum*) used for seasoning, is one of the known therapeutic plant belongs to Amaryllidaceae family, used as health supplements (Bhandari, 2012). Natural products such as ginger, garlic, are used along with herbal medicine which are assumed to have beneficiary effect on human health. Microbial infections and other diseases are treated with natural products and its combinations in pure or crude forms are widely practiced throughout the world (Parekh and Chanda, 2007).

Most of the plants are used to control microbial infections and in antimicrobial therapy due to the secondary metabolites synthesized during growth. Therefore, these extracts and products are subjected to research for

understanding their properties, safety and efficacy (Prusti et al., 2008) Investigating plant based materials against pathogenic fungi through screening is an important attempt to find new bioactive compounds. The study of these extracts, its structure and mode of action has led to prepare potential and lifesaving drugs. Recently, due to the poor management of antibiotics, multidrug resistance has emerged, demanding for new drug (Bhattacharjee et al., 2005). There is an increase dependence on the drugs derived from natural sources for treatment of some infections (Falodun et al., 2006). Interestingly, none of these extracts are used as antibiotics commercially; however, 80% of these products are used in conventional medicine in developed countries. Numerous studies have been carried out for proving the competencies of plant extracts to be used as antimicrobial agents (Prusti, 2008).

Candida albicans a normal microflora, which exists as a commensal in mucocutaneous cavities, vagina and intestine in human (Kaufman, 1997), becomes infectious under altered physiological and pathological conditions (Kennedy et al., 2000). The repeated use of antibiotics can result in development of multidrug resistant organisms or an adverse effect on non-targeted organisms. Using natural products will have less side effects and toxicity with higher bioavailability (Vagionas et al., 2007). Increase in the use and mis-management of antibiotics have a high impact on multidrug resistance and there is an urgent need for safe treatment options. As a result, the uses of natural products or growth inhibitors are gaining attention to treat microbial infections (Kim et al., 2006; Lim et al., 2007).

The operations of cellular components, genetic system and enzyme systems can be altered or interfered by plant metabolites (Kim et al., 2006). Plant constituents have been reported to have antimicrobial properties when tested individually and in different combinations (Ultee et al., 1998). A strong and rapid development is needed in undertaking natural products for the replacement of synthetic materials such as antioxidants and antimicrobial ages (Burt 2004; Sacchetti et al., 2005). Antibacterial, antioxidant and immune stimulating activities of garlic has been revealed. However, only few reports are on antifungal properties, along with some additives. One such additive used in natural medicine is honey. Moreover, honey is used in ancient medicines for nutritional and medical purposes. Another additive used in natural medicine is lemon juice extracted from *Citrus limon* belonging to the family Rutaceae, which has been reported to possess antimicrobial, antiseptic, antihistamine, astringent, detoxifying and anti-inflammatory activity. Antifungal activity of lemon juice has been reported in the treatment of athlete's foot disease. Unique biological and structural components make lemon juice a strong antimicrobial agent (Matasyoh et al., 2007). However, there is no reports of the antifungal activity of lemon along with other combinations. Therefore, the present study was conducted to evaluate the antifungal activities of lemon, honey along with garlic and ginger extracts.

MATERIALS AND METHODS

Chemicals and reagents

This study was conducted at the Microbiology laboratory, College of Applied Medical Sciences Shaqra University, Shaqra, Kingdom of Saudi Arabia. Fresh garlic, lemon and honey were obtained from the local supermarket, Shaqra. Fungal cultures were obtained from the culture collection available at microbiology laboratory, College of Applied Medical Sciences, Shaqra. Yeasts were maintained on Sabouraud dextrose agar (SDA), (Hi-media -India) at 4°C and sub cultures were performed prior to each experiment in the same medium for 48 h at 35°C. Sabouraud dextrose agar (SDA) was suspended (65 g) in one liter of distilled water in a conical flask. The content was heated with frequent agitation and boiled for one minute for dissolving the medium. The pH was adjusted to 5.5 and autoclaved at 121ºC for 15 minutes. After autoclaving the medium was allowed to cool to 40ºC and 15 -20 ml was transferred into sterile Petri plates. The medium was solidified and then stored in refrigerator for future use.

Preparation of extract

Garlic (*A. sativum*) and Ginger (*Z. officinale*) were peeled, squeezed and then extracted by using five layers of cheese cloth. The extract was then filtered and the stock solution was obtained. Various dilutions were made with sterile deionized water. Honey and Lemon juice were obtained in crude form.

Preparation of inocula

Candida spp. was sub-cultured on SDA plates. Colonies were inoculated into sterile broth and incubated at 35 ºC for 24-36 hours. Stock fungal inoculums were prepared in normal saline and the suspension was adjusted to 0.5 McFarland turbidity standards. These

suspensions were inoculated on SDA to determine the colony forming units and determined as 1 x 10^7 - 5 x 10^7CFU/ml (Patton et al., 2006).

Antifungal activity

Agar well diffusion method was adopted for screening the antifungal activity. Sabouraud dextrose agar was inoculated by swabbing the *Candida* suspension using a sterile cotton swab. The plates were kept for 3 min in order to allow the excess moisture to be absorbed into agar. Wells were made on agar by using sterile 6 mm cork-borer. Test concentrations of extracts and its different combinations were added to the wells. The plates were incubated at 35°C for 24 hours to allow the growth of *Candida*. Inhibition zones observed around the wells were measured in millimeters (Perez et al., 1990). Different combinations of test extracts were made as follows: *A. sativum* and lemon, *A. sativum* and honey, Honey and lemon, *Z. officinale* and lemon, *Z. officinale* and honey *Z. officinale* and *A. sativum* at acobination of 1:1. Other combinations were *A. sativum*, lemon and honey, *Z. officinale,* lemon and honey at a ratio of 1:1:1. Transparent zone or inhibitory zone was measured by using a finely calibrated ruler for all of the plates tested.

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of different extracts and its combination was studied by broth dilution test. The tubes containing liquid broth were inoculated with *Candida* spp*.* along with different concentration of extracts ranging from 10^{-1} to 10^{-8}. The tubes were incubated at 37°C for 24 hours and tested for growth by inoculating into SDA plates. The minimum concentration of extracts or its combination which prevents the growth of yeast is calculated as MIC.

Phytochemical analysis of *Z. officinale* and *A. sativum*

The phytochemical analysis of *Z. officinale* and *A. sativum* were determined by standard procedures (Sulaiman et al., 2014). For determination of alkaloids present in the extracts Mayer's test was carried out. For tannins and phenolic compounds, ferric chloride test; alkaline reagent test for flavonoids, froth test for saponins, Brontrager's test for glycosides, for steroids Lieberman's test were used. For terpenoids, Salkowski test and general tests were carried out for carotenoids, anthraquinones, oxalates and phytates.

RESULTS

The present study on the antifungal activity of ginger and garlic extract along with some combinations showed that *Candida albicans* growth has been inhibited by ginger extract (19 mm) and garlic (21 mm) respectively with 100% crude extract. With low concentration of crude extracts (75%) the fungal growth was also inhibited (Table 1). Meanwhile the other two species of *Candida* showed less susceptibility to the 100% extracts. The inhibition of fungal growth was dose dependent, where the least concentration (25%) did not show any much inhibitory activity. There are reports on extensive drug resistance of opportunistic pathogens towards some of the antifungal drugs such as azoles (Whaley et al., 2016). As the *Candida* is getting resistance to antifungal agents it is necessary to elucidate new drug for treating fungal infections. However, in the present study, ginger and garlic extract will provide an alternative choice of controlling the *Candida* infections. Various therapeutic effects of ginger have been reported which includes anti-emetic activity, anti-ulcer, anti-platelet, antipyretic, anti-inflammatory and antioxidant activity (Lemar et al., 2002). Isolation of novel antimicrobial component has been focused to overcome multidrug resistance. Some of these materials are having great potential due to its availability, less toxicity, lack of adverse effects and easily degradability (Vagionas et al., 2007). Garlic has been used as natural antibiotic for animals as well as antifungal activity of garlic has been reported (Ledezma and Apitz-Castro, 2006). These products which are isolated naturally can be used for food preservation to offer potential health benefits (Akobundu and Agyakwa, 1987; Misra et al., 1992; Hablemarian et al., 1993; Davidson, 2006). Some of the compounds extracted from plants have been used to control pathogens (Corthout, 1992). Large number of sulphur compounds present in garlic are responsible for the antifungal activity (Supreetha et al., 2011). Around 33 different sulphur containing compounds are present in garlic including water soluble ones such as *S*-allylcysteine, and oil soluble ones such as allicin (allyl 2-propenethiosulfinate) (Bhandari, 2012).

Table 1; Antifungal activity of aqueous extracts of ginger and garlic against *Candida* spp.

Spices	Concentration (%)	Zone of inhibition (mm)*		
		Candida albicans	***Candida krusei***	***Candida tropicalis***
Ginger (Zingiber officinale)	100	19	15	14
	75	16	11	12
	50	13	9	8
	25	6	6	6
Garlic (Allium sativum)	100	21	14	16
	75	18	12	12
	50	15	9	8
	25	8	6	6

*values are mean of three replicates

Table 2; Synergetic Antifungal activity of different combinations of garlic extract, ginger extract, honey and lemon juice against *Candida* spp.

*Extracts /Combinations	Concentrations/ ratio	Zone of inhibition (mm)*		
		Candida albicans	***Candida krusei***	***Candida tropicalis***
Lemon	100%	6	6	6
Honey	100%	6	6	6
***A. sativum* and lemon**	1:1	23	19	18
***A. sativum* and honey**	1:1	24	17	20
Honey and lemon	1:1	6	6	6
***A. sativum*, lemon and honey**	1:1:1	25	18	20
***Z. officinale* and lemon**	1:1	21	17	17
***Z. officinale* and honey**	1:1	23	19	18
***Z. officinale* lemon and honey**	1:1:1	24	18	20
Z. officinale* and *A. sativum	1:1	25	23	22

For different combinations 100% of the extracts were used
*values are mean of three replicates

Allicin is a sulphur containing compound released during the crushing of garlic is also reported to possess antimicrobial activity (Ledezma and Apitz-Castro, 2006).

Allicin has been reported to have antifungal activity *in-vitro* and *in-vivo* which include *Candida* spp., *Cryptococcus* spp., *Trichophyton* spp.and *Aspergillius* spp. (Lemar et al., 2002). Allicin has been reported to have antimicrobial activity against bacterial strains such as *Escherichia coli* and *Staphyloccocus aureus*, and fungal strains *C. albicans* and *Trichophyton rubrum* (Corthout et al., 1992; Al-Waili, 2005).

Antimicrobial properties of lemon and honey have attracted the interest of researchers working on novel drugs to control multidrug resistant microorganisms. In the present study the inhibitory activities of lemon juice and honey were significantly less, as compared to garlic and ginger when tested individually. There are several possible reasons which may be due to the less antifungal effect against *Candida* or diffusion rates on agar plates due to different molecular weight which may contribute to variations in the inhibitory zones. Antibacterial activity of both natural products such as honey and lemon juice has been reported earlier. For inhibiting the growth of *S. aureus* was 50% of honey was added in the media and 66% of honey concentration is used for inhibiting *C. albicans* in Sabouraud glucose agar-honey mixture media (Banaeian-Borujeni et al., 2013). Honey has been reported for the treatment of vaginal candidiasis without any antimicrobial effect on normal flora (Canonico, 2014). Flavonoids present in honey prevent the yeast hyphal transition and also inhibit reactive oxygen species (ROS) and gamma-glutamyl transpeptidase (GGT) activity in the host cells. It also supports intracellular GSH level. Utilization of honey in ancient medicine is popular and termed as api-therapy used in the treatment of bacterial infections.

In the present study the mixture of garlic and honey showed maximum inhibition zone (24mm) against *Candida* sp. whereas, garlic and lemon, and the other combination of garlic, lemon and honey showed inhibitory activity (25 mm). Honey and lemon combinations have less or no inhibitory

activity (6 mm) against *Candida* sp. (Table 2). It should also be noted that the anti-*Candida* activity of the garlic with lemon and honey, used in this study, has not been reported earlier. Antifungal activity of garlic alone was less than Garlic honey and Garlic lemon juice against *Candida*. This may explain that the antifungal activity of these mixtures have potent fungicidal effects as compared to garlic. So it is clear that garlic has a synergistic effect when it combines with other components.

Ginger and lemon combinations were tested for antifungal activity against *Candida albicans* and found that it inhibited the growth of the pathogen (21mm). Other combination of ginger extract with honey showed 23mm inhibitory activity. Ginger extract with lemon and honey were also showed inhibitory activity against the pathogen. Because of its non-toxicity and natural bacteriostatic properties, ginger is widely used to control infections. The study indicates that ginger extract might have promise in the treatment of candidial infections with other combinations of drugs. However further research is needed *in-vivo* as well as *in-vitro* to reach a better conclusion. Ginger has been reported to have antifungal effect. The components such as gingerol and shogaol have been identified in ginger to possess antimicrobial effect (Atai et al., 2009). Inhibitory activity of ginger on oral microflora has been reported earlier (Park et al., 2008). Antivirulence effect and antibiofilm formation of components extracted from ginger on *Candida albicans* was also studied earlier (Lee et al., 2018).

The minimum inhibitory concentration (MIC) of different extracts was studied. Lemon and honey was needed in a higher concentration to inhibit the growth of *Candida albicans*. There was no much difference in the inhibitory effect with honey and lemon combinations. The growth of *Candida* was inhibited with garlic honey combination and garlic lemon combinations. The combination of lemon and honey with garlic has significant effect on antifungal activity. The effect of ginger extract with different combination has been studied. The combination of lemon and honey along with ginger extract had more activity than lemon and honey when used individually with ginger (Table 3).

Table 3; Minimum Inhibitory Concentration (MIC) of extracts and its different combinations

*Extracts /Combinations	Minimum Inhibitory Concentrations		
	Candida albicans	***Candida krusei***	***Candida tropicalis***
Lemon	10^{-1}	--	--
Honey	10^{-1}	--	--
A. sativum and lemon	10^{-3}	10^{-1}	10^{-1}
A. sativum and honey	10^{-3}	10^{-2}	10^{-2}
Honey and lemon	10^{-1}	--	--
A. sativum, lemon and honey	10^{-3}	10^{-2}	10^{-2}
Z. officinale and lemon	10^{-2}	10^{-1}	10^{-1}
Z. officinale and honey	10^{-2}	10^{-1}	10^{-1}
Z. officinale lemon and honey	10^{-3}	10^{-1}	10^{-1}
Z. officinale and *A. sativum*	10^{-3}	10^{-2}	10^{-2}

-- Not determined

Table 4; Phyto chemical analysis of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*)

Components	***Allium sativum***	***Zingiber officinale***
Alkaloids	+	+
Saponins	+	+
Cardiac glycosides	+	+
Steroids	+	+
Tannins	-	+
Flavanoids	+	+
Carotenoids	+	+
Phenolics	+	+
Terpenoids	+	+
Antraquinone	+	+
Oxalates	+	+
Phytates	+	+

+ =present; - = absent

Moreover, ginger and garlic extract also showed significant anti-fungal activity against *Candida albicans*. The inhibitory activity of spices has been reported earlier. It is widely used for the preservation and increasing the self-life of food materials (Gull et al, 2012). The use of such spices in the daily diet may reduce the side effects of allopathic drugs as well as the development of antibiotic resistance.

The extracts used in the present study were subjected to phytochemical analysis and the compounds present were alkaloids, saponins, cardiac glycosides, steroids, flavanoiods, carotenoids, phenolic components, terpenoids, antraquinone, oxalates and phytates (Table 4). However, tannins were not detected in garlic extracts. Organosulphurate compounds such as allicin, ajoene and thiosulfinates present in garlic are known for antifungal properties (Ledezma and Apitz-Castro, 2002). Garlic, lemon and honey are most efficient with antifungal activity at the anticipatory level; it can be suggested for daily use. The pungent smell of garlic makes patient to hesitate it to use regularly. For overcoming the smell, the additives such as honey and lemon can be used.

CONCLUSION

Natural plant based components are a major source of antimicrobial drugs and with less side effects. It is inexpensive, better patient tolerance and readily available for the weaker socioeconomic populations. Finally, it can be concluded that due to increasing resistance of current and old antibiotics, it may be suggested that the use of plant based drugs to overcome the resistance developed by microorganisms.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Dr. AAAG designed the work and reviewed the manuscript; Dr. BJ and BS carried out the laboratory works; Prof. MSA contributed for preparation and revision of the manuscript.

REFERENCES

Akobundu, I.O. and Agyakwa, C.N. 1987. A handbook of West African Weeds. International Institute of Tropical Agriculture. 240: 272-348.

Al-Waili, N.S. 2005. Mixture of honey, beeswax and olive oil inhibits growth of *Staphylococcus aureus* and *Candida albicans*. Archives of Medical Research. 36(1): 10-13.

Atai, Z., Atapour, M. and Mohseni, M. 2009. Inhibitory effect of Ginger Extract on *Candida Albicans*. Am. Journal of Applied Science. 6(6): 1067-1069.

Banaeian-Borujeni, S., Mobini, G.R., Pourgheysari, B. and Validi, M. 2013. Comparison of the effect of honey and miconazole against *Candida albicans* in vitro. Advances in Biomedical Research. 30(2): 57.

Bhandari, P. 2012. Garlic (*Allium sativum* L.): a review of potential therapeutic applications International Journal of Green Pharmacy. 6: 118-129.

Bhattacharjee, I., Ghosh, A. and Chandra, G. 2005. Antimicrobial activity of the essential oil of *Cestrum diurnum* (L.) (Solanales: Solanaceae). African Journal Biotechnology. 4: 371-374.

Burt, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology. 94: 223–253.

Canonico, B., Candiracci, M., Citterio, B., Curci, R., Squarzoni, S., Mazzoni, A., et al. 2014. Honey flavonoids inhibit *Candida albicans* morphogenesis by affecting DNA behavior and mitochondrial function. Future Microbiology. 9: 445-456.

Corthout, J., Pieters, L.A., Claeys, M., Vanden-Berghe, D.A. and Viletinck, A.J. 1992. Antiviral caffeoyl: esters from *Spondias mombin*. Phytochemistry. 31: 79-81.

Davidson, P.M. 2006. Food antimicrobials: back to nature. In: Frenkel, D.C., Dudai, N. (Eds.),

Proceedings of the First International Symposium on Natural Preservatives in Food Systems. International Society for Horticultural Science, Belgium. pp. 29–33.

Falodun, A., Okunrobo, L.O. and Uzoamaka, N. 2006. Phytochemical screening and anti-inflammatory evaluation of methanolic and aqueous extracts of *Euphorbia heterophylla* Linn (Euphorbiaceae). African Journal Biotechnology. 5: 529-531.

Gull, I., Saeed, M., Shaukat, H., Aslam, S.M., Samra, Z.Q. and Athar, A.M. 2012. Inhibitory effect of *Allium sativum* and *Zingiber officinale* extracts on clinically important drug resistant pathogenic bacteria. Annals of Clinical Microbiology and Antimicrobials. 11: 8.

Hablemarian, S., Gray, A.I. and Waterman, P.G. 1993. A new antibacterial sesquiterpene from *Premma oligotrichia.* Journal of Natural Products. 56: 140-143.

Kaufman, H.K. 1997. Opportunistic fungal infections: Superficial and systemic candidiasis. Geriatrics, 52: 50-54.

Kennedy, W.A., Laurier, C., Gautrin, D., Ghezzo, H. and Pare, M.J.L. 2000 Contandriopoulos AP: Occurrence of bad risk factors of oral candidiasis treated with oral antifungals in seniors using inhaled steroids. Journal of Clinical Epidemiology. 53: 696–701.

Kim, H.G., Cho, J.H., Jeong, E.Y., Lim, J.H., Lee, S.H. and Lee, H.S. 2006. Growth inhibiting activity of active component isolated from *Terminalia chebula* fruits against intestinal bacteria. Journal of Food Protection. 69(9): 2205–2209.

Kim, J., Marshall, M.R. and Wei, C.I. 1995. Antibacterial activity of some essential oil components against five foodborne pathogens. Journal of Agriculture and Food Chemistry. 43: 2839-2845.

Ledezma, E. and Apitz-Castro, R. 2006. Ajoene the main active compound of garlic (*Allium sativum*): a new antifungal agent. Revista Iberoamericana de Micología. 23:75–80.

Lee, J.H., Kim, Y.G., Choi, P., Ham, J., Park, J.G. and Lee, J. 2018. Antibiofilm and antivirulence activities of 6-gingerol and 6-shogaol against *Candida albicans* due to hyphal inhibition. Frontiers in Cellular and Infection Microbiology. 28(8): 299.

Lemar, K.M., Turner, M.P. and Lloyd, D. 2002. Garlic (*Allium sativum*) as an anti-*Candida* agent: a comparison of the efficacy of fresh garlic and freeze-dried extracts. Journal of Applied Microbiology. 93(3): 398-405.

Lim, M.Y., Jeon, J.H., Jeong, E.Y., Lee, C.H. and Lee, H.S. 2007. Antimicrobial activity of 5-hydroxy-1,4-naphthoquinone isolated from *Caesalpinia sappan* toward intestinal bacteria. Food Chemistry. 100: 1254–1258.

Matasyoh, L.G., Matasyoh, J.C., Wachira, F.N., Kinyua, M.G., Muigai, A.W. and Mukiama, T.K. 2007. Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya African Journal of Biotechnology. 6: 760-765

Misra, T.N., Singh, R.S., Pandey, N.S., Prasal, C. and Singh, B.P. 1992. Antifungal essential oil and a long chain alcohol from *Achyranthis aspera*. Phytochemistry. 31: 1811-1812.

Parekh, J. and Chanda, S. 2007. *In vitro* antibacterial activity of crude methanol extract of *Woodfordia fruticosa* Kurz flower (Lythacease). Brazilian Journal of Microbiology. 38: 2.

Park, M., Bae, J. and Lee, D.S. 2008. Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. Phytotherapy Research. 22: 1446–1449.

Patton, T., Barrett, J., Brennan, J. and Moran, N. 2006. Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. Journal of Microbiology Methods. 64: 84-95.

Perez, C., Pauli, M. and Bazerque, P. 1990. An antibiotic assay by agar well diffusion method. Acta Biologiae et Medicinae Experimentalis. 15: 113-115.

Prusti, A., Mishra, S.R., Sahoo, S. and Mishra, S.K. 2008. Antibacterial activity of some Indian medicinal plants. Ethnobotanical Leaflets. 12: 227-230.

Sacchetti, G., Maietti, S., Muzzoli M., Scaglianti, M., Manfredini, S. and Radice, M. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry. 91: 621–632.

Sulaiman, F.A., Kazeem, Mo., Waheed, A.M., Temowo, S.O., Azeez, I.O., Zubair, F..I., et al. 2014. Antimicrobial and toxic potential of aqueous extracts of *Allium sativum Hibiscus sabdariffa* and *Zingiberofficinale* in Wistar rats. Journal of Taibah University for

Sciences. 8: 315–322.
Supreetha, S., Sharadadevi, M., Sequeira, P.S., Jithesh, J., Shreyas, T. and Amit, M. 2011. Antifungal activity of ginger extract on *Candida albicans*: An *In-vitro* study. Journal of Dental Sciences and Research. 2: 18-21.
Ultee, A., Gorris, L.G. and Smit, E.J. 1998. Bactericidal activity of carvacrol towards the food-borne pathogen *Bacillus cereus*. Journal Applied Microbiology. 85: 211–218.
Vagionas, K., Ngassapa, O., Runyoro, D., Graikou, K., Gortzi, O. and Chinou, I. 2007. Chemical analysis of edible aromatic plants growing in Tanzania. Food Chemistry. 105(4): 1711–1717.
Whaley, S.G., Berkow, E.L., Rybak, J.M., Nishimoto, A.T., Barker, K.S. and Rogers, P.D. 2016. Azole antifungal resistance in *Candida albicans* and emerging non-*albicans Candida* species. Frontiers in Microbiology. 7: 2173.