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Natural Herbs as Alternative to Synthetic Antifungal Drugs- The Future Challenging Therapy

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ABSTRACT

Background: Oral Candidiasis which is due to overgrowth of candida species especially candida albicans, is mostly encountered among all. The use of natural antimicrobials such as Punica granatum and lawsonia innermis have been an alternate and effective way of treating oral candidiasis because of large scale resistance and adverse reactions of the commercially available azole group of synthetic antifungal drugs.

Aim: This study evaluates the antifungal properties of pomegranate (punica granatum) peel extract, and Henna leaves (*Lawsonia innermis*) with different solvents such as aqueous, ethanol, methanol and chloroform in different concentrations i.e., 25%, 50% and 75% against pure colonies of candida albicans.

Materials and methods: Extracts of punica granatum peel, and lawsonia innermis leaves were prepared and their anticandidal properties were determined. Then the minimum zones of inhibitions in millimeters expressed by both the plant extracts were measured and compared.

Results: Among all the solvents and concentrations used, at 25% concentration punica granatum methanol showed the maximum zone of inhibition (**mean zone=13.6**), at 50% concentration punica granatum chloroform showed maximum inhibition zone (**mean zone=15.4mm**) and finally at 75% lawsonia chloroform showed the maximum inhibition zone(**mean zone=17.4mm**).

Conclusion: The extracts of Punica granatum peel and Lawsonia innermis leaves showed significant zones of inhibition against candida albicans and hence can be used as a cost effective way of treating oral candidiasis with no adverse effects in comparison to synthetic anti fungal drugs.



Introduction

Infectious diseases have become world's leading cause of premature deaths, almost killing approximately fifty thousand people per day. In recent years, resistance to human pathogenic organisms has been frequently reported from all over the world¹. However situation is alarming in both developing as well as developed countries due to indiscriminate use of antibiotics. The treatment of infectious diseases in immune-compromised patients has become further complicated due to the resistance of bacterial and fungal pathogens.¹

Most common fungal infections such as candidiasis are caused by yeast like fungus² *Candida albicans*, a dimorphic fungus³ that exists in three forms namely, pseudohyphae, yeast, and chlamydospore forms. These species grow rapidly at 25-37°C temperature.² *Candida albicans* colonizes mucosal surfaces of the oral and vaginal cavities and the digestive tract and is also able to cause variety of infections depending upon the nature of the underlying host defect³. Weak or immature immune system or metabolic illness such as diabetes, HIV/AIDS, stress, nutrient deficiency, mononucleosis are important predisposing factors for candidal infections².

Discovery of antibiotics to combat these infections marked a resolution⁴. The Synthetic antifungal drugs act mainly by exploiting the Ergosterol, a cell membrane sterol, found in the fungi by reducing the Ergosterol synthesis by inhibition of fungal cytochrome p450 enzymes⁵. But their inappropriate use, blocks the action of antibiotics and change their target or ability to penetrate cells⁴ with adverse effects like, renal damage, abnormalities of liver function, gastrointestinal upset⁵.

In the present scenario emergence of multiple drug resistant human pathogenic organism, has necessitated the search for new antimicrobial substances from other sources such as plants and fruits. *Punica*

granatum and *Lawsonia inermis* have proven their antifungal efficacy which can offer viable alternative in terms of cost, efficient in action and less toxic module without any side-effects.

Pomegranate is an ancient fruit⁶ that has been widely approved for their antimicrobial property⁷. Phytochemically peel extract of pomegranate contains active antifungal compounds like Punicalagin, Castgalagin, Granatin, catechin, Gallocatechin, kaempferol, and quercetin⁶. The synergistic interactions of these compounds might increase the antifungal activity of pomegranate peel extract.

Herbal medicines have been known to all for centuries. The henna plant *Lawsonia inermis* being one such plant is attributed to antibacterial, antifungal, antiamebiasis, astringent, antihemorrhagic, hypotensive, and sedative effect⁴. The plant leaf contains lawsone (2-hydroxy-1, 4-Naphthoquinone) about 0.5 to 1.5% of its ingredients and is the main constituent responsible for the dyeing properties. However, henna also contains mannite, tannic acid, mucilage and gallic acid.⁸

The present study is just a venture from the usual clinical approach. The use of fruit and medicinal plants against *Candida* can be a feasible alternative to other antifungal agents as these offer inexpensive and effective module. The lack of pharmacological and chemical data of *Punica granatum* and *Lawsonia inermis* prompted investigation into their antifungal activity against *Candida albicans*.

Materials and Methods

The present in-vitro experimental study was carried out by using fruit *Punica granatum* (pomegranate peel) and herbal plant *Lawsonia inermis* (henna leaves) on *Candida albicans* (MTCC 3017) which were obtained from Microbial Type Collection

Centre and Gene Bank (Institute of Microbial Technology, Chandigarh) (**Figure 1**). The study was conducted in the Department of Oral Pathology and Microbiology, Kothiwal Dental College and Research Centre, Moradabad, U.P. Ethical clearance was also obtained from institutional ethical committee, Kothiwal Dental College and Research Centre.

Preparation of extract

The peels of punica granatum (pomegranate) and leaves of lawsonia innermis (henna) were obtained from juice corners and botanical garden respectively in Moradabad city. Both the peel and leaves were sundried, then grinded manually using mortar and pestle to obtain fine powder and sieved to get rid of any impurities. The extract of different concentration 25%, 50% and 75% was prepared by mixing 25gms, 50gms, and 75gms of powder into 100ml of deionized distilled water, ethanol, chloroform and methanol separately and the mixture was soaked, and kept in dark for 24hours. After 24hrs the mixtures of different concentration in different agents was subjected to filter by using filter paper to remove any impurities and was stored at 4°C.

Preparation of culture media for Candida albicans

20.5gm of malt yeast agar was suspended in 490ml of distilled water and heated to boiling to dissolve the medium completely. Sterilization was done by autoclaving at 15LBS pressure (121°C) for 15minutes and mixed well and then was poured into sterile petri plates and stored until use.

Preparation of cultures plates for the study

The ampoules containing freeze dried pure forms of the candida albicans were opened and the contents were added to

normal saline. A sterile swab was dipped into the contents and then incubated at 37°C for 24 hours on malt yeast agar plate for candida albicans. The growth obtained on the agar plate was transferred on their respective plates by streaking using an inoculation loop having an internal diameter of 0.4mm. The streaking was done by streak method for isolation of pure candida albicans. Thereafter, the plates were incubated for the isolation of candida albicans for 24 hours at 37°C for antifungal activity of punica granatum & lawsonia innermis extract. Following incubation, PAS staining was also done for preliminary confirmation of the Candida albicans.

Assay of antifungal activity

An antifungal activity of the prepared extracts of different concentrations was assessed by using agar well diffusion method. 5mm diameter of wells was prepared by using punch and different concentrations of extract and control (clotrimazole 1%) were used accordingly. The test organisms Candida albicans MTCC 3017 were gently removed with pointed top of ampoule and 0.3 to 0.4 ml of specific medium was added to make a suspension of the culture. Few drops of test organism's suspension were streaked on malt yeast agar in Petri plates and incubated at 25°C for 48 hours (**Figure 2a & figure 2b**). Diameter of the inhibitory zones was measured to the nearest millimeter by using inhibition zone scale and the measurements were noted after 48hours (**Figure 3**).

Results

The present study evaluated the antifungal activities of pomegranate peel extract (rind), and Henna leaves (Lawsonia innermis) against candida albicans. Aqueous, Ethanolic, Methanol and Chloroform extracts of pomegranate peel and henna leaves were prepared. Anti-candidal activity

was determined and minimum zones of inhibitions (in mm) shown by extracts of both herbs were measured and compared.

Table 1, 2, & 3 reveals comparison of antifungal efficacy of Punica Granatum & Lawsonia Innermis in different concentrations and in different solvents with Control and **Graph 1, 2 & 3** shows the means of average zone of inhibition for candida albicans of Punica Granatum & Lawsonia Innermis in different concentrations and in different solvents and control. Among all the solvents and concentrations used, at 25% concentration, punica granatum methanol showed the maximum zone of inhibition (**mean zone=13.6**), at 50% concentration, punica granatum chloroform showed maximum inhibition zone (**mean zone=15.4mm**) and at 75%, lawsonia chloroform showed the maximum inhibition zone (**mean zone=17.4mm**).

Discussion

Inspite of enormous achievements regarding oral health of populations worldwide, problems still continue in many communities especially among low socio economic groups in developing countries.⁹

Candida being a normal oral commensal may show quite notorious properties under favorable environment which may lead to discomfort for patients. 30% to 50% of people simply carry this organism in their mouth without any clinical evidence of infection¹⁰ and this increase in colonization of candida in oral cavity can predispose to chronic hyperplastic candidiasis, median rhomboid glossitis, (thrush), and angular cheilitis (perleche).¹¹

There is an immense interest in the search for new antimicrobial agents of plant origin due to increase of antibiotic resistance as well as adverse effects of synthetic drugs and also due to resistance to azole

antifungals which was reported in 1980s after prolonged therapy with miconazole and ketoconazole.¹²

The present study showed the antifungal effect of punica granatum and lawsonia innermis in different concentrations. Results of the antifungal screening of different solvent extracts of both the herbs revealed significant antifungal activity against candida albicans.

Regarding the mechanism of action of henna, it may be due to presence of mucilage mannite, tannic acid and gallic acid which are in the form of a mixture. Antimicrobial activity is due to free hydroxyls that have ability to combine with proteins and carbohydrates in the bacterial cell wall and get attached to enzyme sites making them inactive.¹³ However still the mode of action of lawsone is still not very clearly understood, and more studies should be done on large scale to determine how lawsonia compounds affect the organism.¹⁴

In punica granatum, punicalgin is mainly responsible for antifungal activity¹⁵. Punicalgin is absent in the seeds of pomegranate, the reason why in the present study punica granatum peels were used to assess antifungal effect. Although mechanism of action of Punicalgin is also not much clear but it may be due to their astringent, toxicity, molecular structure which might be responsible for antifungal properties.¹⁶

In present study malt yeast agar was used as growth medium for candida albicans MTCC 3017 as it contains fungi static materials such as sodium propionate and diphenyl in order to eliminate moulds and thus permits enumeration of yeasts from mixed population. Also it contains Peptic digest of animal tissue which serves as a source of essential nutrients, carbon and nitrogen. Yeast extract provides vitamin B complex nutrients and other growth factors and malt extract acts as an additional source

of carbon while dextrose is the carbohydrate and energy source.¹⁷

According to **M.A. Abdulmoneim Saadabi(2007)⁴** and **Muhammad and Muhammad(2005)¹⁸** there is a general rule that any plant is considered active against fungi and bacteria when the zone of inhibition is greater than **6mm** thus supporting our results, which are more than **6mm**, at every different concentrations and in different solvents.

In the present study as the solvent concentration decreased and punica granatum and lawsonia concentration in particular percentage increased, the zone of inhibition also increased, proving again antimicrobial properties of both natural antifungals.

Regarding the safe dose of lawsonia innermis that can be used in patients it was stated by **P. Panichayupakaranant and W. Reanmongkol (2002)¹⁹** that maximum safe dose that can be applied in oral cavity is **1gm** of preparation which corresponded to **5mg lawsone methyl ether oral base** in their study.

Conclusion

The extracts of Punica granatum peel and Lawsonia innermis leaves showed significant zones of inhibition against candida albicans and hence can be used as a cost effective way of treating oral candidiasis. The use of various medicinal herbs and plants as substitutes of antifungal drugs against oral candidiasis have been found to be effective thereby overcoming the adverse effects of the known antifungal drugs such as clotrimazole. Further studies are definitely required to support the herbal line of treatment for management of oral candidiasis. Since natural antifungal agents like punica granatum and lawsonia innermis have many advantages yet they are not commonly used by patients due to unavailability of these herbs in

commercially available oral antifungals. So, it is advisable to use the fine extract of these herbs in mouth washes or in oral ointments so that patients will be benefitted. Finally it is also recommended that these natural antimicrobials be used with aqueous solvent as it might have almost negligible side effects as compared to other solvents such as ethanol, chloroform and methanol.

Conflict of interest

We declare that we have no conflict of interest.

References

1. Iqbal Ahmad, Arina Z. Beg. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J Ethnopharmacol*2001; 74: 113-123.
2. R Rajendran, B Sivapathasundharam, Shafer's Textbook of Oral Pathology. 6th edition Elsevier; 2009. p.363.
3. Gloria et al. Candida Albicans: Genetic dimorphism and pathogenicity. *International Microbiology*1998; 1: 95-106.
4. M.A Abdulmoneim Saadabi. Evaluation of Lawsonia innermis Linn. (Sudanese Henna) Leaf Extracts as an anti microbial agents. *Research Journal Of Biological Sciences*2007; 2(4): 419-423.
5. Bertran G. katzug. Basic and clinical pharmacology, 8th edition, 2001.p814-817.
6. Saad Sabber Dahham, Mir Naiman Ali, Hajera Tabassum, Mazhrudin khan. Studies on antibacterial and antifungal activity of pomegranate (Punica granatum L.) *American-Eurasian Journal of Agriculture & Environmental Science*2010; 9(3): 273-281.
7. Mithun BH Pai, Prashant GM, Murlikrishna KS, Shivakumar KM, Chandu GN. Antifungal efficacy of punica granatum, Acacia nilotica, Cuminum cyminum and Foeniculum vulgare on Candida albicans. *Indian J Dent Research*2010; 21(3): 334-336.
8. Kathem k. Al-rubiay, Nawres N Jaber, Al-Mhaawe BH, laith k Alrubaiy. Antimicrobial

- efficacy of henna extract. *Oman Med J* 2008; 23(4): October.
9. Hiremath S.S Singh Gurumukh. Caries preventive effect on early carious lesions of fluoride varnish and fluoride containing mouth rinse in 12-14 year old school children in Bangalore city. *Archives of oral science and research* 2011;1(2):50-59.)
 10. Parihar S. Oral Candidiasis- A Review. *Webmed Central Dentistry*.2011;2(11):1-18.
 11. Jeyanthi Premkumar. Detection of species diversity in oral candida colonization and anti-fungal susceptibility among non-oral habit adult diabetic patients. *Journal of Natural Science, Biology and Medicine* January 2014 .Vol 5,Issue 1.
 12. Casalnuovo IA, DI Francesco P, Garaci E. Fluconazole resistance in *Candida albicans*: a review of mechanisms. *Eur Rev Med Pharmacol*.2004;8:69-77.
 13. Al-Rubiay KK, Jaber NN, Al-Mhaawe BH, Alrubaiy LK. Antimicrobial Efficacy of Henna Extracts. *Oman Med J*. 2008Oct;23(4):253-6.
 14. Sritairat N, Nukul N, Inthasame P, Sansuk A, Prasirt J, Leewatthanakorn. Antifungal activity of lawsone methyl ether in comparison with chlorhexidine. *J Oral Pathol Med*. 2011;40:90–6.
 15. Hoda P, Hassanpur S, Emam-djome Z, Lavasani AS. Antimicrobial properties of Pomegranate (*Punica granatum L.*) as a tannin rich fruit: a review. The 1th International and the 4th National Congress on Recycling of Organic Waste in Agriculture 26 – 27 April 2012 in Isfahan, Iran
 16. Sh. Abdollahzade, Mashouf RY, Mortazavi H, Moghaddam MH, Roozbahani N, Vahedi M. Antibacterial and antifungal activities of *Punica Granatum* peel extracts against oral pathogens. *J Dent*. 2011;8(1):1-6.
 17. Himedia technical data 2011.
 18. H.S Muhammad and S. Muhammad. The use of lawsonia inermis linn(henna) in the management of burn wound infections. *African Journal Of Biotechnology*. 2005;4(9). 934-937
 19. P. Panichayupakaranant and W. Reanmongkol. Evaluation of chemical stability and skin irritation of lawsone methyl ether in oral base. *Pharmaceutical Biology*. 2002;40(6). 429-432.

Table 1: Comparison of antifungal efficacy of 25% Punica Granatum & 25% Lawsonia Innermis in different solvents with Control

	Mean	N	Std. Deviation	Std. Error Mean	t value	d.f.	p value
PG Aqueous	11.60	5	2.074	.927	4.792	4	.009*
Control	23.00	5	6.442	2.881			
LI Aqueous	11.60	5	1.140	.510	3.508	4	.025*
Control	23.00	5	6.442	2.881			
PG Ethanol	13.00	5	1.225	.548	3.536	4	.024*
Control	23.00	5	6.442	2.881			
LI Ethanol	11.80	5	1.924	.860	4.251	4	.013*
Control	23.00	5	6.442	2.881			
PG Methanol	15.00	5	4.000	1.789	4.159	4	.014*
Control	23.00	5	6.442	2.881			
LI Methanol	12.80	5	2.588	1.158	3.127	4	.035*
Control	23.00	5	6.442	2.881			
PG Chloroform	13.60	5	3.209	1.435	2.642	4	.057**
Control	23.00	5	6.442	2.881			
LI Chloroform	11.20	5	1.643	.735	4.188	4	.014*
Control	23.00	5	6.442	2.881			

* Significant ($p < 0.05$), ** Not Significant ($p > 0.05$)

Table 2: Comparison of antifungal efficacy of 50% Punica Granatum & 50% Lawsonia Innermis in different solvents with Control

	Mean	N	Std. Deviation	Std. Error Mean	t value	d.f.	p value
PG Aqueous	15.20	5	2.683	1.200	2.786	4	.050**
Control	23.00	5	6.442	2.881			
LI Aqueous	12.20	5	1.789	.800	3.126	4	.035*
Control	23.00	5	6.442	2.881			
PG Ethanol	14.80	5	1.304	.583	2.874	4	.045*
Control	23.00	5	6.442	2.881			
LI Ethanol	13.40	5	1.673	.748	3.121	4	.035*
Control	23.00	5	6.442	2.881			
PG Methanol	14.00	5	2.550	1.140	3.503	4	.025*
Control	23.00	5	6.442	2.881			
LI Methanol	14.20	5	4.438	1.985	3.047	4	.038*
Control	23.00	5	6.442	2.881			
PG Chloroform	15.40	5	3.130	1.400	2.119	4	.101**
Control	23.00	5	6.442	2.881			
LI Chloroform	14.20	5	2.775	1.241	3.143	4	.035*
Control	23.00	5	6.442	2.881			

* Significant ($p < 0.05$), ** Not Significant ($p > 0.05$)

Table 3: Comparison of antifungal efficacy of 75% Punica Granatum & 75% Lawsonia Innermis in different solvents with Control

	Mean	N	Std. Deviation	Std. Error Mean	t value	d.f.	p value
PG Aqueous	16.00	5	2.550	1.140	2.320	4	.081**
Control	23.00	5	6.442	2.881			
LI Aqueous	13.20	5	1.924	.860	3.062	4	.038*
Control	23.00	5	6.442	2.881			
PG Ethanol	16.00	5	2.550	1.140	2.490	4	.067**
Control	23.00	5	6.442	2.881			
LI Ethanol	14.80	5	3.033	1.356	2.165	4	.096**
Control	23.00	5	6.442	2.881			
PG Methanol	16.00	5	2.000	.894	2.523	4	.065**
Control	23.00	5	6.442	2.881			
LI Methanol	15.40	5	3.362	1.503	2.317	4	.081**
Control	23.00	5	6.442	2.881			
PG Chloroform	16.80	5	3.834	1.715	1.599	4	.185**
Control	23.00	5	6.442	2.881			
LI Chloroform	17.40	5	5.079	2.272	1.187	4	.301**
Control	23.00	5	6.442	2.881			

* Significant ($p < 0.05$), ** Not Significant ($p > 0.05$)



Fig. 1: Candida albicans fungal strains 3017 used in study



Fig. 2a: Pure colonies of candida albicans after 48 hours.

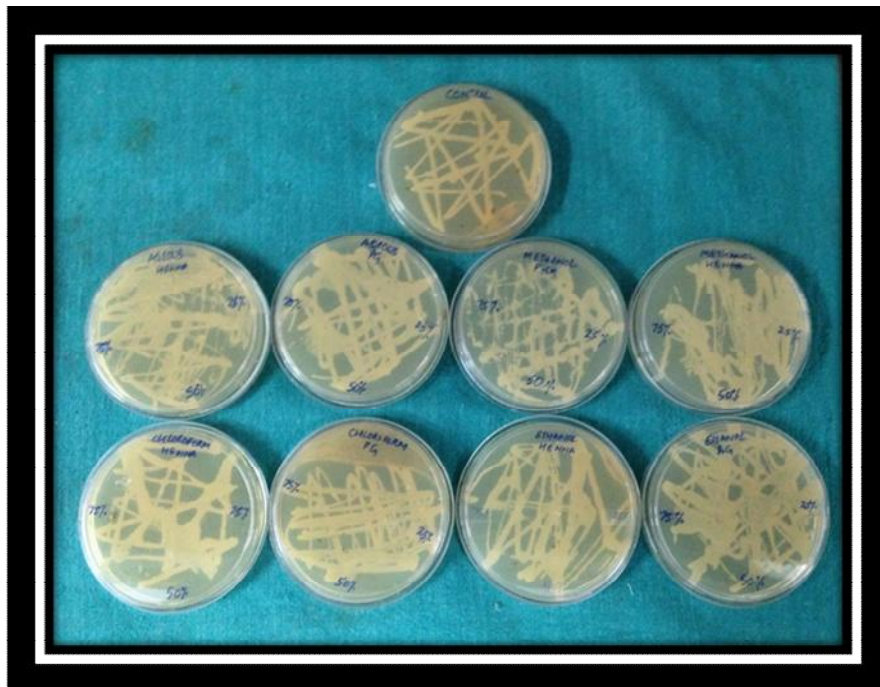


Fig. 2b: Pure colonies of candida albicans after 48 hours of incubation.

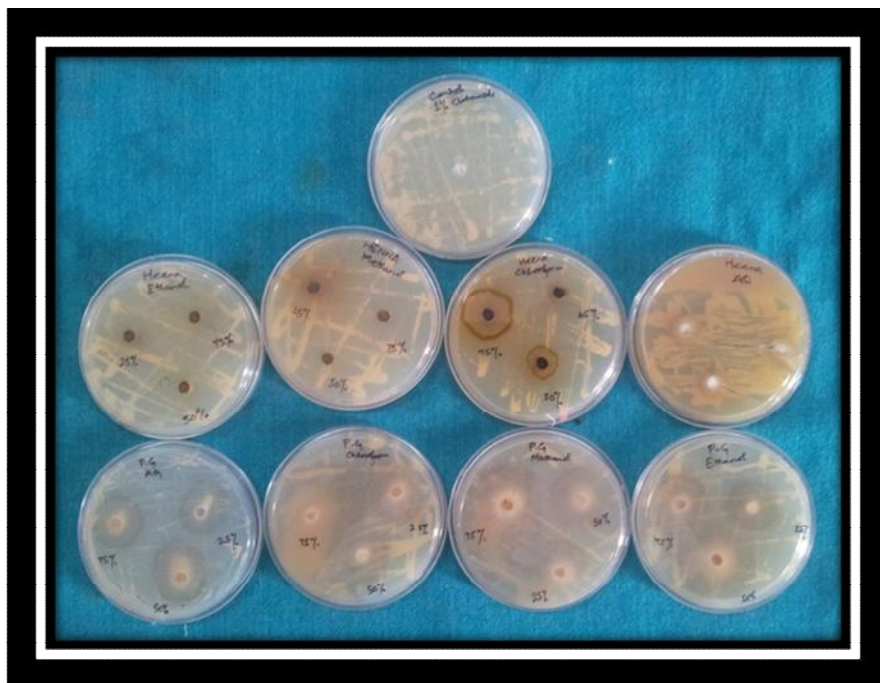
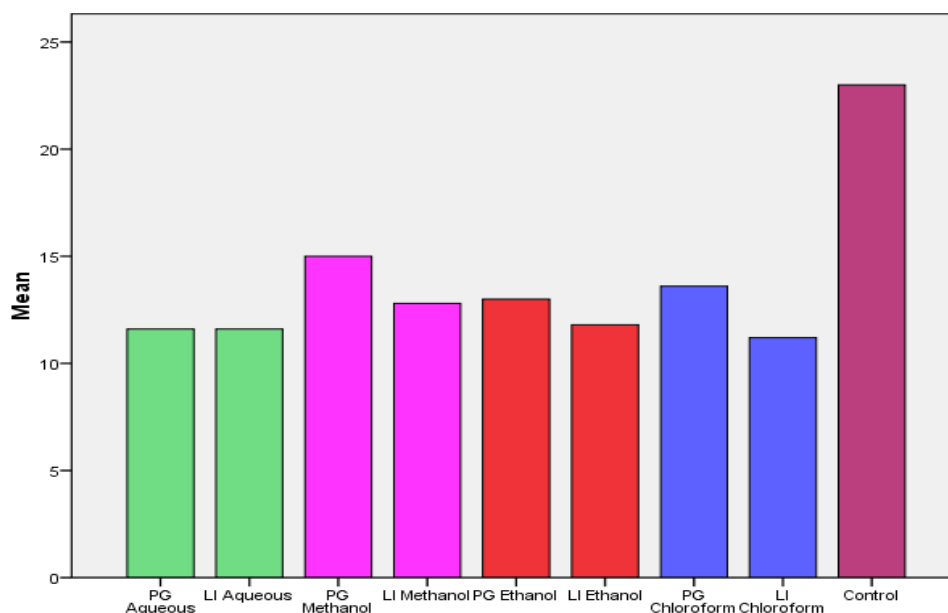
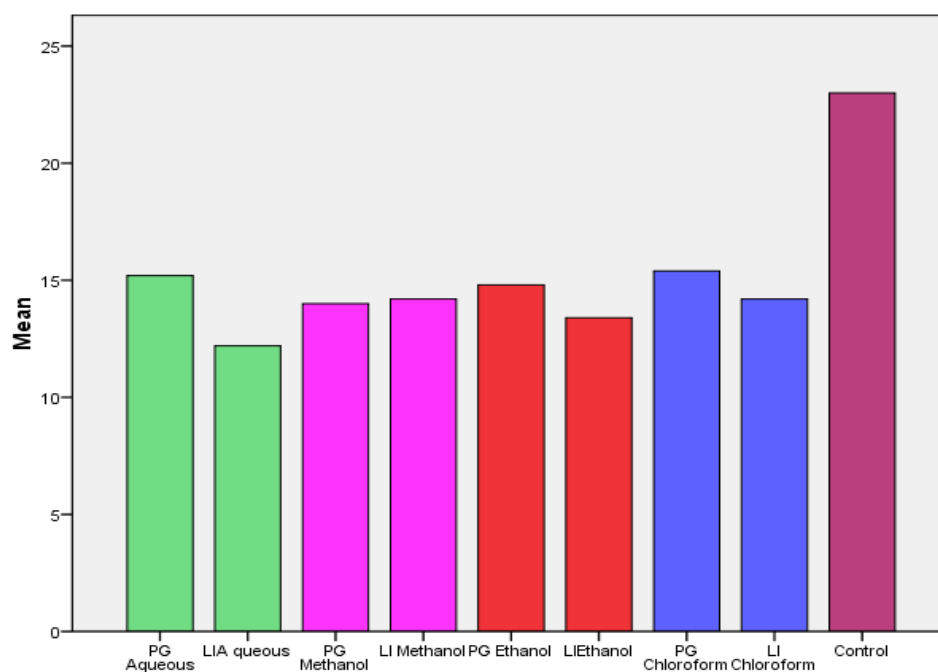


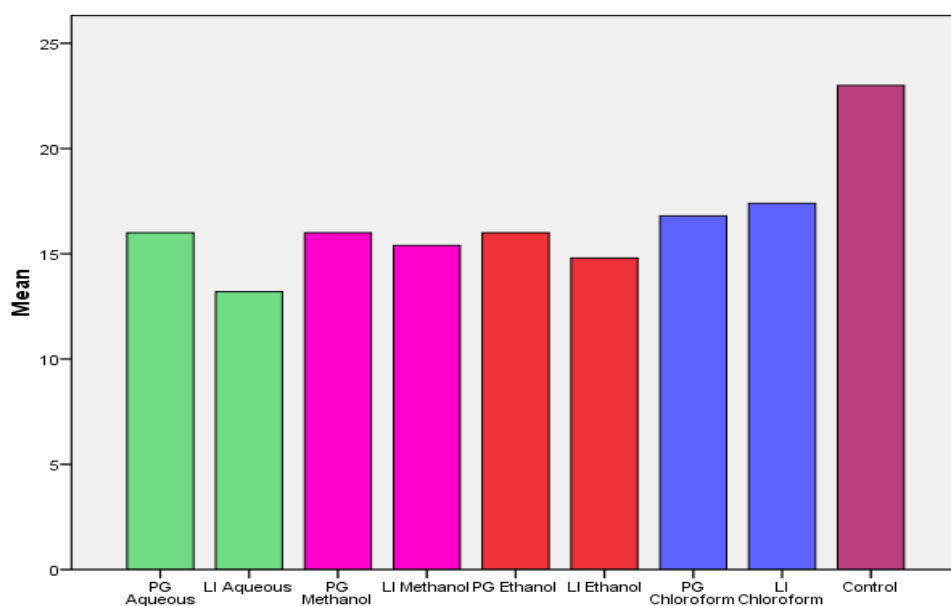
Fig. 3: Zones of inhibition of both plant extracts at 48 hours.



Graph 1: The means of Average zone of inhibition in millimeters for candida albicans of 25% Punica Granatum & 25% Lawsonia Innermis in different solvent and control



Graph 2: The means of Average zone of inhibition in millimeters for candida albicans of 50% Punica Granatum & 50% Lawsonia Innermis in different solvent and control



Graph 3: The means of Average zone of inhibition in millimeters for candida albicans of 75% Punica Granatum & 75% Lawsonia Innermis in different solvent and control