5. DISCUSSION

Health, as per World Health Organization (WHO) in 1946, is defined as "a condition encompassing total physical, psychological and communal, and not simply the lack of ailment or illness”. Global population of Oral health problems still remain in many communities around the world particularly among underprivileged groups in developed and developing countries. India is no exception to these problems and they are widely prevalent in India too.

The overgrowth of usually benign yeast *Candida* is now becoming a recognized infection known as oral candidiasis. Oral candidiasis is an opportunistic mycotic infection of the oral cavity and patients with oral candidiasis report sensations of oral burning, altered taste, dysphagia from esophageal overgrowth resulting in poor nutrition, bad breath, pain at the corners of the lips and generalized or localized mucosal soreness with or without a maxillary denture, local discomfort, slow recovery, and prolonged hospital stay. It is manifested in a diversity of clinical forms ranging from mucocutaneous infection to a life-threatening systemic disease.

Mycological studies have shown that *C. albicans* represents over 80% of isolates from all forms of human candidiasis. However, the so-called non-*Candida albicans* Candida species are increasingly recognized as important agents of human infection (Williams et al., 2000). The two *Candida* species viz., *C. tropicalis* and *C. krusei* constitute the majority of clinically significant yeasts in most of the clinical cases. The polymorphic fungus *Candida* is normally a harmless commensal of host epithelial tissues, but it can cause life-threatening systemic infections in individuals with impaired immune systems, in whom the mortality rate from infection exceeds 30% (Sexton, Brown & Johnston, 2007).

Changes in the oral environment that can predispose or precipitate oral candidiasis include: antibiotics, corticosteroids, dry mouth (xerostomia), diabetes mellitus, nutritional deficiencies, and immunosuppressive diseases and therapy (Madhu priya, 2013). The expanding population of immunocompromised patients those use intravenous catheters, total parenteral nutrition, invasive procedures and the increasing use of broad-spectrum antibiotics, cytotoxic chemotherapies and transplantation are factors that contribute to the increase of these infections. When an overgrowth of organism occurs, it becomes a local irritant that can produce an acute inflammatory reaction causing local edema and the formation of microabscesses, which can put the host at risk for the loss of integrity of the underlying epithelial surface. In fact, the critical event for the development of candidiasis occurs when
the microorganism actually penetrates the mucosal surface (Sudbery et al., 2004). Once the epithelial surface has been traumatized, *Candida* can penetrate the mucosal barrier easily and bypass the natural defense mechanism, leading to the establishment of infection.

The pathogenicity of *Candida* species is attributed to certain virulence factors, such as the ability to evade host defences, adherence, biofilm formation (on host tissue and on medical devices) and the production of tissue-damaging hydrolytic enzymes such as proteases, phospholipases and haemolysin. Currently, an increase in the number of yeasts that are resistant to antifungal drugs is recognized worldwide. Oral candidiasis causes many people to experience a great deal of continuous discomfort through impaired function and as well as inconvenient treatment.

As a result of these, the research community insisting them to look for an effective alternative. When the immune system is not able to generate an adequate quantity of antibodies, it is possible to use antibodies via passive immunization (Larsson et al., 1993). Antibodies generated from chicken egg yolk against *Candida* have been acknowledged as a cost-effective substitute antibody source (Ibrahim et al., 2008; Takeuchi et al., 2014). The use of antigen-specific chicken egg yolk antibodies can be considered as one such approach.

Chicken egg yolk antibodies (IgY) has considerable attention for preventing and controlling disease as it holds a large number of advantages compared with mammalian IgG including cost-effectiveness, convenience and high yield (Carlander et al., 2000). Under natural conditions, the serum IgY of laying hens is being transferred in large quantities in the egg yolk in order to protect the developing embryo from potential pathogens. Chicken egg yolk has been recognized as an inexpensive alternative antibody source, and passive immunization with egg yolk immunoglobulin (IgY) has shown therapeutic value. This raises the possibility of conferring passive protection against *Candida albicans*, *C. tropicalis*, *C. krusei*-induced oral candidiasis by using antibody prepared from the eggs of the hyperimmunized chicken.

Hence, the present investigation was aimed to generate chicken egg yolk antibodies against three antigens of *Candida albicans*, *Candida tropicalis* and *Candida krusei* and to test their potential both *in vitro* and *in vivo* to control the virulence properties of the yeast. These antibodies were analysed for their ability to effectively block the adherence of *Candida* and prevent the colonization, thus inhibiting and preventing the development of oral candidiasis.

### 5.1. Generation and characterization of Anti-*Candida* sp IgY in white leghorn chickens:

The standard strain of *Candida albicans* (MTCC 227), *Candida tropicalis* (MTCC 184) and *Candida krusei* (MTCC 9215) was procured from Microbial Type Culture
Collection center and characterized based on its morphological, cultural and biochemical characteristics to confirm its purity (Parihar, 2011). *Candida* sp., antigens were prepared by sonication as per the method prescribed by Ibrahim *et al.* (2008). It was also confirmed that the prepared antigen was pure and sterile with their corresponding specificity by plating on SDA and gram staining. Twenty-one weeks old white leghorn chickens were immunized with antigen (5×10⁶ CFU/ml) of *C. albicans*, *C. tropicalis* and *C. krusei* respectively. Booster doses were given at 14 days interval. The eggs were collected periodically and stored at 4°C.

There were many possible factors which may influence the egg production in the regular layer farms, but during antibody generation there was one more possible factor which was suspected to affect the laying capacity of chickens, that was immunization with the prepared antigens. Hence, the egg laying performance of immunized chickens were monitored for 25 weeks from the date of first immunization. It was found that the immunization with the antigens did not influence egg production and the egg laying capacity of chickens were influenced by factors other than immunization. Similar observation with other antigens was reported by Schade *et al.*, (1994).

The specific antibody level in chicken serum and the titre of anti-*C. albicans* IgY, anti-*C. tropicalis* IgY, anti-*C. Kruse* IgY in 1000-fold dilution of IgY-extracts obtained from the eggs of laying hens immunized with antigens were determined by Indirect ELISA using the antigen and mentioned as optical density (OD) at 405nm (ELISA value). An increase in antibodies against three *Candida* sp., was detected in the serum of immunized chicken 7 days after the initial immunization by ELISA. This humoral immunity reached a peak at about 45 days. However, in the egg yolk, the antibody level was increased 2 weeks after the initial immunization and persisted increase was observed till the 56th day after which the antibody level remained stable till the 160th day of immunization. The titre of specific antibody was found to be 1:50000 on 56th Day and the titre were maintained with booster doses. These results were comparable to the work done by O’Farrelly *et al.*, 1992, which showed that antibodies started to appear in serum 10 days after immunization began and reached high titre at 45th day and remained stable till 168th day observation. This long lasting titre of antibodies correlated with the results Fugybhyasi *et al.*, 2009, have shown a good correlation between ELISA and the potency of IgY raised against *Candida*. To this end, although both primary and secondary immunization induced a high titter of antigen-specific IgY antibody in the egg yolk, induction of *Candida* sp., antibody was noted two weeks later, when compared with serum antibody, which is similar to the findings as noted by Kitaguchi *et al.*, 2008.
Egg yolk antibodies were purified from immunized eggs by PEG extraction method prescribed by Polson et al., (1980). IgY extract was then further purified by dialysis and DEAE cellulose column chromatography. The DEAE cellulose column purified antibody samples were analysed for their purity by SDS-PAGE technique. A high molecular weight protein (180KD) was detected by using Coomassie brilliant blue stain. It shows the purity of egg yolk antibodies. The total protein content of the chicken egg yolk was detected by Lowry’s method of protein estimation. The protein concentration of the egg yolk from the eggs of the 21 weeks old immunized chicken was found to be 22.34 ± 0.05 mg/ml. This concentration of protein increased slowly and reached a steady level giving a maximum content of 40.49 ± 0.17 mg/ml, 41.16± 0.67 mg/ml and 41.16 ± 0.71 mg/ml for anti- C.albicans IgY, anti- C.tropicalis IgY and anti-C.krusei IgY respectively at the 160th day after immunization. This increase in protein concentration was due to the increase in the age of the bird, as well as the increase in the egg yolk volume. This correlated with the findings of Li et al., 1998. Similarly, the total IgY concentration was estimated photometrically at 280nm (1:50 diluted with PBS) with the extinction coefficient of 1.33 for IgY (Pauly et al., 2011). The total IgY concentration of egg yolk was~8.34 ± 0.10mg/ml of egg yolk during the 7th day of immunization after which there was a steady increase in the concentration to reach a maximum of 22.71± 0.19 mg/ml, 22.84 ± 0.05 mg/ml and 22.71 ± 0.19 mg/ml for anti- C.albicans IgY, anti- C.tropicalis IgY and anti-C.krusei IgY respectively. This result was in correlation with the results of Rose et al., 1974 where the total IgY concentration in the egg yolk was in the range of 10-25mg/ml. Moreover, the results showed that irrespective of the antigen used the amount of total IgY in the egg yolk had no considerable variation. This was in association with the findings reported by Lee et al., 2002, which indicated the total IgY concentration was independent of the type of antigens used to generate antibodies.

The reactivity of the specific IgY in the egg yolk was studied using RSA test for anti- Candida sp., IgY and using Indirect ELISA for anti- C.albicans IgY, anti- C.tropicalis IgY and anti-C.krusei IgY. Agglutination of these antigens on assay plate confirmed the derived IgY were specific to Candida sp., antigen used. Similarly, when pre-immune IgY (control) were used no agglutination were observed. Likewise, in ELISA a peak titre was observed even at a dilution of 1:50000 in immunized chicken egg yolk against anti- C.albicans IgY, anti-C.tropicalis IgY and anti- C.krusei antigen during 160th day of observation.

IgY purified from both the immunized and unimmunized eggs was tested for their specificity reaction against their respective antigens. It was found that there was no
significant increase in the titre of specific IgY in unimmunized egg yolk when compared to immunized egg yolk. The specific antibody titre in the immunized egg yolk was significantly higher than the un-immunized egg yolk. This indicated that the production of specific IgY could be efficiently elicited in chickens using simple protocols of immunization and extraction, this finding was comparable with the report of Guimaraes et al., 2009.

The stability of IgY when incubated at different conditions was assessed by determining the difference between the specific reactivity of treated IgY and untreated control IgY using ELISA. Specific reactivity of IgY (conc. 0.5mg/ml) after being treated at different temperatures (4ºC, 25ºC, 37ºC, 60ºC, 70ºC, 80ºC and 90ºC) for 30 minutes estimated by ELISA revealed that the IgY solution retained its stability at 4ºC, 25ºC, 37ºC and 60 ºC. At 70ºC there was a significant decrease in the antibody activity and a complete loss of activity was observed at 80ºC and 90ºC. The pH stability study showed that the IgY was stable at pH ranges between 4 and 9. There was significant decrease in the activity of IgY at pH 2 and a slight decrease inactivity at pH 3 and 10. A complete loss of antibody activity was observed at pH 12 when compared to that of the untreated control IgY. Similar results were reported by Jaradat & Marquardt, (2000).

5.2. Evaluation of neutralization potential in vitro

Cytotoxic effect of organism was performed on KB cell lines (HeLa contaminant cell line. Stereo zoom microscopic analysis of KB cell lines treated with IgY showed little or no significant changes in the cell morphology with increased concentration. The control cells used in the study showed 100% viability. The anti-CA IgY at 2.5 mg concentration showed cell viability of 100%, at 5 mg concentration it showed 105.2% and at 10 mg the cell viability was 155.4%. Similarly, anti-CT IgY at 2.5 mg concentration showed cell viability of 103%, at 5 mg concentration cell viability was 102% and at 10 mg it was 158% and anti-CK shows 98% at 2.5 mg concentration, at 5 mg concentration cell viability was 107% and at 10 mg it was 154%. The concentration of IgY increases can enhance the proliferation of lymphocytes. Lymphocytosis is a late cellular response and is related to the production of specific and lasting antibodies. Antibodies are a major opsonins produced by the host defense system and they are essential for the stimulation of antigen phagocytosis (Riley T et al., 2008). The results revealed the non-cytotoxic nature of Candida sp, as the cells were viable at different concentrations and are compared with Sampio et al., 2014.

The anti-C. albicans, anti-C. tropicalis, anti-C. krusei IgY was tested for its efficacy to inhibit the cell growth of C. albicans, C. tropicalis, C. krusei by in-vitro method. The Growth
curve of *Candida* sp in liquid medium was plotted until the stationary phase reached at OD 660nm using a spectrophotometer. Then with similar conditions, the growth of *C.albicans*, *C.tropicalis* and *C.krusei* with the anti-*C.albicans* IgY, anti-*C.tropicalis* and anti-*C.krusei* respectively was observed by growth inhibition assay. The growth of *C.albicans*, *C.tropicalis*, *C.krusei* with specific IgY showed significant reduction when compared with unimmunized IgY. The inhibition of cell growth was noted to be increased with the increase in the duration of incubation. The results were comparable with reports given by Fujibayashi *et al.* (2009).

An *in vitro* adhesion inhibition assay was performed to investigate the effect of the three anti-*Candida* sp IgY on the adherence of *C.albicans*, *C.tropicalis*, *C.krusei* to monolayers of KB cells. The three anti-*Candida* sp IgY significantly reduced (*P* <0.05) the adherence capacity of *C.albicans*, *C.tropicalis*, *C.krusei* to monolayers of KB cell lines when compared to the non-treated sample (cell lines not treated with organism). But no significant reduction was observed in control IgY-treated *Candida* sp., Similarly they showed significant inhibition in (*P* <0.05) in dose-dependent manner (0 to 5 mg). At 0 mg/ml concentration the viable count was 3.2×10^5 CFU/ml whereas at 5 mg/ml it was 8.1×10^2 CFU/ml. There is a direct correlation between the *C. albicans* adhesion inhibition and IgY concentration. For *C.tropicalis* inhibition at 0 mg/ml concentration the viable count was 8.1×10^4 CFU/ml whereas at 5 mg/ml it was 1.52×10^3 CFU/ml and for *C.krusei* inhibition at 0 mg/ml concentration the viable count was 1.6×10^5 CFU/ml whereas at 5 mg/ml it was 1.2×10^3 CFU/ml. The results revealed that there is a direct correlation between the *Candida* adhesion inhibition and IgY concentration. Similar findings were observed by Ibrahim *et al.* (2008). These antibodies were found to possess the ability to reduce the adhesion properties of *Candida* possibly by altering the cell wall adhesions or by blocking the adherence of *Candida* to the host cells as well as the difficulty of organism in invading tissues. Interrupting with the adherence of a particular pathogen prevents or delays the colonization since adherence is the essential condition for exhibiting the virulence of the mucosal pathogens and for the establishment of the disease. In addition, agglutination caused by IgY when binds with the yeast cells, results in the reduction of the number of independent infection unit which could be another possible mechanism (Ibrahim *et al.*, 2008; Fujibayashi *et al.*, 2009).
5.3. Determine the neutralization effects of anti-\textit{Candida} \textit{sp} IgY in experimental mice model induced oral candidiasis

SPF, female, Balb/c mice (n=24) were used to perform the experiment and the mice were assigned to 5 experimental groups (n=6 mice). The experimental group 1 consists of animals infected with \textit{C.albicans} and treated with anti-\textit{C.albicans} IgY until the end of the experiment; group 2 consists of animals infected with \textit{C.tropicalis} and treated with anti-\textit{C.tropicalis} IgY until the end of the experiment; group 3 was infected with \textit{C.krusei} and treated with anti-\textit{C.krusei} IgY; group 4 was infected with all the three species and treated with consortium of antibodies and group 5 was infected and treated with control IgY.

The body weight of mice was recorded from day 1 to 7. All the mice groups expressed weight loss after injection with prednisolone. The group 1 treated with anti-\textit{C.albicans} IgY showed a gradual decrease in the body weight after the third prednisolone injection. The weight loss observed was 1.14±0.03 gm at day 1 and increased to 3.6±0.02 gm at day 5 after infection. They experienced maximum weight loss was at day 5. Then at day 6 (3.21±0.03 g) and day 7 (3.05±0.04 g) they slowly started to regain their body weight. The group 2 treated with anti-\textit{C.tropicalis} IgY showed weight loss of about 1.1±0.02 g at day 1 and 3.34±0.04 g at day 5. They started to gain weight at day 6 (3.34±0.04 g) and day 7 (3.1±0.03 g). The group 3 treated with anti-\textit{C.krusei} IgY showed weight loss of about 1.18±0.02 g at day 1 and 3.46±0.02 g at day 5. They started to gain weight at day 6 (3.32±0.05 g) and day 7 (3.2±0.03 g). The weight loss was in the consortium group started from day 1 (1.02±0.04 g) after the third prednisolone injection and reached 3.2±0.01 g at day 5. They regained their body weight at day 6 (3.16±0.02 g) and day 7 (3.02±0.02 g). On the other hand, severe weight loss was observed in the control IgY group from day 1 to day 7 and the animals did not regain their body weight. No significant differences of the relative body weight gain/loss were observed between the first three groups in the statistical analysis was compared with result described by Ibrahim \textit{et al.} (2008). Statistical analysis showed significant differences in the body weight of the control IgY treated group in comparison with the infected groups.

The progression of infection was macroscopically evaluated by lesions scoring on the tongue of experimental mice. The lesion scoring was recorded and tabulated at days 1, 3, 5, and 7 after infection, based on the severity and the number of the white patches on the tongue. At day 1 after infection, no lesions were observed in anti-\textit{C.albicans} IgY, anti-\textit{C.tropicalis} IgY, anti-\textit{C.krusei} IgY, consortium and unimmunized IgY treated groups. In the
anti-*C. albicans* IgY group, lesion score of 1±0.01 was recorded at day 3 after infection. They showed mild lesions with the lesion score of 0.3±0.01 at day 7 after infection, which was significantly decreased score when compared with unimmunized IgY treated group which showed lesion score at day 3 (2.3±0.06) and at day 7 (1.3±0.04). In group 2, lesion score of 1.3±0.02 was recorded at day 3 after infection. They showed mild lesions with the lesion score of 0.6±0.01 at day 7 after infection. In group 3, lesion score of 1.6±0.03 was recorded at day 3 after infection. They showed mild lesions with the lesion score of 0.6±0.01 at day 7 after infection. Similarly, mice treated with consortium showed lesion score of 2.6±0.12 at day 3 after infection and score 0.3±0.01 at day 7 which was also significantly decreased lesion score when compared with the control IgY treated group. Statistical analysis showed significant differences (*P* < 0.05) in the score of consortium group in the comparison with the individual treatment groups.

Oral swabs were collected on days 1, 3, 5 and 7 after infection for enumeration of viable cells of *C. albicans, C. tropicalis, and C. krusei*. The initial colonization in the control IgY treated group was 4.3×10^5 CFU/ml at day 1 and it reached 9.45×10^5 CFU/ml at day 7. In group treated with anti-*C. albicans* IgY, initial colonization was 4.36×10^5 CFU/ml with the final count of 1.6×10^5 CFU/ml. In group 2 treated with anti-*C. tropicalis* IgY, initial colonization was 5.1×10^5 CFU/ml with the final count of 1.9×10^5 CFU/ml. In group 3 treated with anti-*C. krusei* IgY, initial colonization was 4.9×10^5 CFU/ml with the final count of 1.5×10^5 CFU/ml which were significantly (*P* < 0.05) less than the control IgY treated group. In group 4 treated with consortium, initial colonization was 5.3×10^5 CFU/ml with the final count of 0.7×10^5 CFU/ml which was also highly significant (*P* < 0.05) than control IgY group. The results were compared with Takakura et al. (2003), 10^5–10^6 CFU of *Candida* from each murine oral cavity was recovered continuously each day until day 7 after inoculation. Correlating with the fungal burden, white patches consisting of the hyphae were macroscopically observed on the dorsal tongues and germ tube formation was microscopically shown on the tongue surface. The severity of *Candida* infection was estimated by manifestation scores of these white tongue patches and by CFU of *Candida* in the oral cavity.

Histopathological examination and fungal detection were done using Periodic Acid-Schiff staining of the tongue sections taken from sacrificed animals. Microscopic observation (40X) of the typical lesion on the surface of the tongue on day 3 showed spores and extensive colonization of *Candida* on the epithelium of the dorsal surface of the tongue. Microscopic observation on day 7, showed reduced in *Candida* spores and shows normal lingual papillae.
on the stratified squamous epithelium of the tongue; group 2 showed histological lingual candidiasis on the tongue surface on day 3, day 7 shown fewer spores and normal lingual papillae on the squamous epithelium of the tongue; in group 3 pseudo hyphae and normal lingual papilla was observed on day 3 and on day 7 fewer spores were observed; group 4 shown group of hyphae in the layers of epithelium on day 3, and on day 7 only spores were observed where as control IgY treated group showed spores and hyphae of Candida attached to the squamous epithelium on day 7. This result was in correlation with reports given by Takakura et al. (2003).

The disseminated infection was evaluated from the kidney homogenates of the experimental mice since kidney is a target organ in disseminated candidiasis (Han and Cutler, 1995). Serial dilution of the kidney homogenates was performed and plating was done on HiChrome agar for Candida (HiMedia, India). The viable count was expressed as CFU/g tissue, and determined after 48 hours of incubation at 37°C. A viable count of $1.11 \times 10^5$ CFU/g of tissue was recovered from anti-C. albicans IgY treated group; $1.23 \times 10^5$ CFU/g of tissue was obtained from anti-C. tropicalis IgY treated group; $1.2 \times 10^5$ CFU/g of tissue was obtained from anti-C. krusei IgY treated group; $1.41 \times 10^5$ CFU/g of tissue was obtained from consortium group at day 3 whereas $1.09 \times 10^5, 1.16 \times 10^5, 1.19 \times 10^5$ and $1.03 \times 10^5$ CFU/g of tissue respectively was recovered at day 7 after infection. On the other hand, from control IgY group, at all points of examination Candida was recovered from all mice. The statistical analysis showed that Candida sp was recovered in increased rate at days 3, 5 and 7 after infection from kidneys of control IgY treated mice and there was a significant ($P < 0.05$) difference in the recovered CFUs/g between the first 4 groups and control group. The results obtained in the experimental mice model are in correlation with the findings of Ibrahim et al. (2008).

5.4. **In vitro evaluation of commercially available chemical mouth rinses and formulated test mouth rinses**

Mouth rinses are widely used as adjuncts to oral hygiene and in the delivery of active agents to the oral cavity. The effectiveness of commercially available mouth rinses against Candida albicans, C. tropicalis, C. krusei was screened by agar diffusion method (MithunPai et al., 2013). Minimal inhibitory concentration (MIC) of the mouth rinses was determined by the broth dilution method. The lowest dilution at which growth of Candida inhibited was recognized as the minimum inhibitory concentration. For C. albicans, formulation A has the MIC at the dilutions of 1:64; formulation C shows MIC at concentration of 1:64 for
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C. tropicalis and for C. krusei formulation C shows MIC at 1:64. The results indicated that all the tested mouth rinses were efficient on inhibition of Candida sp., growth. The results of the present study were similar as reported by MithunPai et al. (2013).

The antimicrobial activity of the tested mouth rinses against C. albicans, C. tropicalis and C. krusei had shown varying degrees of zones of inhibition. Among all the investigated mouth rinses, mouth rinses A showed highest anti-Candidal activity. This might be due to the presence of Chlorhexidine gluconate in its formulation. It is a cationic biguanide which acts by binding to the negatively charged cell walls of the microbes; subvert their osmotic balance results in inhibition of growth and cell death (Nagappan and Joseph, 2012). Though it is considered as the gold standard among other mouth rinses, it is also reported to have a number of side effects, such as taste alteration, discoloration of teeth and tongue, excruciating mucosa and gingival desquamation. Next to mouth rinse A, mouth rinse D, B and C were found to have antimicrobial activities against Candida.

The active ingredient in mouth rinse D is cetyl pyridinium chloride (CPC). It is a cationic quaternary ammonium compound. Its mechanism of action is by interacting with the cell surface of the microbes, it incorporates into the cytoplasmic membrane. This disrupts the membrane integrity results in an outflow of cytoplasmic constituents thereby inhibit the cell growth and cause cell death. It is evidenced from a study (Giuliana et al., 1999) that CPC showed better antifungal activity than Chlorhexidine. However, it is also reported to cause side effects such as staining of teeth and tongue, distress in taste and sensibility, mouth burning effect and formation of white plaque on the tongue. Mouth rinse B possesses Listerine mouth rinse. In a study conducted by Meiller et al., (2001), the antifungal effect of mouth rinses against different species of Candida was investigated and reported that Listerine antiseptic showed greater efficacy against attached artificial biofilms than other antimicrobials tested. Listerine is an essential oil containing mouthwash with the longest history of use that contains phenolics such as thymol, eucalyptol, menthol, and methyl salicylate. It acts by disrupting the cell wall and inhibiting the bacterial enzymes. They may also able to take out the endotoxin derived from the lipopolysaccharide of gram-negative bacteria (Mhaske et al., 2012). However, use of Listerine may cause burning sensation (Prashant et al., 2013). Moth rinse E contains 3% potassium nitrate which is usually prescribed for its de-sensitizing action and Mouth rinse C containing Povidone Iodine, is a water soluble combination of molecular iodine and the solubilizing agent polyvinyl pyrrolidone both was found to exhibit very weak inhibitory activity against Candida sp.
The prepared mouth rinse formulations containing anti-\textit{C. albicans} IgY, anti-\textit{C. tropicalis} IgY, anti-\textit{C. krusei} IgY and consortium of anti-\textit{Candida} sp., IgY were tested for their antimicrobial activity against \textit{Candida} sp in a liquid medium by broth dilution method using CLSI (2008) protocol. The mouth rinse base used in the formulation without IgY showed 29% reduction of \textit{Candida} growth and considered as the negative control. Positive control was the \textit{Candida} inoculated broth culture containing no mouth rinse, the OD value of this positive control was considered as 100% growth. The test mouth rinses were effective in controlling \textit{Candida} growth and they were able to bring significant reduction in \textit{Candida} growth. The mouth-rinse containing anti-\textit{C. albicans} IgY (1mg/ml) shown 91% reduction of \textit{Candida} growth, anti-\textit{C. tropicalis} IgY (1mg/ml) shown 89%, anti-\textit{C. krusei} IgY (1mg/ml) shown 90% reduction whereas consortium of anti-\textit{Candida} sp IgY (0.5mg/ml) was able to bring a 95% reduction of \textit{Candida} sp., growth. The reduction in growth was expressed in percentage as compared to the positive control.

The results of this study provides a platform for the control of oral candidiasis using antibodies prepared from chicken egg yolk (IgY) against \textit{C. albicans}, \textit{C. tropicalis}, \textit{C. krusei}. It was revealed that by blocking the mode of action of the yeast pathogenicity, it is possible to prevent the colonization of oral candidiasis in experimental mice challenged with the pathogen. These egg yolk antibodies were used for formulation as an oral mouth rinse composition and their activity was assessed. They showed a significant reduction in \textit{Candida} growth. Hence, a novel oral formulation incorporating them as consortium could be developed as an oral composition which will be a reliable, safe, and economic with less or no side effects against Oral Candidiasis, which serves as a prophylactic agent.