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Efficacy of Herbal Control of the Yeasts Isolated from Autistic Children

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Abstract

Autistic children were reported to have gastrointestinal problems more frequent and severe than in normal children. The observation that antifungal medications improve autism behavior, made us aims to investigate their intestinal colonization with yeasts, the risk of yeast overgrowth in autistic behavior and evaluation of the antifungal activity of some plant extracts and essential oils on yeasts isolated from autistic children *in vitro*, 25 cases diagnosed as autistic children were taken as test group and 10 normal children were considered as a control group. Isolated yeasts from autistic children were identified and tested to be inhibited growth by some essential oils and plant extracts through agar well diffusion method. Minimum inhibitory concentration was determined to the clove extract. This study indicated that heavy growth of yeasts in stool culture among autistic children is common features without considering level of autism severity; Clove oil and extract have considerable antifungal activity against isolated yeasts.

***Key words**: Autistic children, Yeast growth, Clove oil and cinnamon extract.*

1. Introduction

Autism spectrum disorders (ASDs) are a group of neurodevelopmental disorders, characterized by retarding in social interactions, communication deficits, restricted, repetitive and stereotyped routine and diagnosed at children before 3 years. Internationally Autistic disorder (ASD) and its related conditions are estimated to affect 10-15 people per 10,000 populations' worldwide (Center for Disease Control and Prevention, 2012). Two decades ago, autism was detected in 1 in 1000 children; as of 2014 an estimated 1 in 88 children has been identified with autism. It is partially explained by improvements in case ascertainment, as well as increasing awareness of the disorder among the general public (Fombonne, 2003; Crawford, **S.** 2016). Exact etiology of autism remains largely unknown, although it is likely to result from a complex combination of environmental, neurological, immunological and genetic factors (Rutter, 2000; Wiznitzer, 2004; Cohen, *et al.*, 2005; Dawson, 2016).

Gastrointestinal (GI) infections are common in autistic patients, in whom symptoms include abdominal pain, bloating, diarrhea and constipation (Horvath, *et al*., 1999; Afzal, *et al*., 2003*)*. No exact percentage explain GI problems in autism, but prospective reports from pediatric gastroenterology and general autism clinics have described GI symptoms in 46– 84% of patients with autism (Horvath, *et al*., 1999).

The reason for the development of such a complex network is to maintain gastrointestinal homeostasis keeping in mind its links with cognitive and affective functions. Recently, the role of enteric flora, or microbiota, has been recognized as a part of the gut–brain axis. The gut microbiota can modulate brain function, forming a crucial link in the bidirectional interactions between the intestine and the nervous system (Laura de Magistris, *et al*., 2016).

Yeast inhabits almost all humans. It lives on the moist dark mucous membranes which line the intestinal tract. Ordinarily it exists only in small amount, prevented from rapid growing by the human immune system, and by natural balance with other microorganisms in and on the body's mucous membranes, but due to autistic children yeast can grow rapidly and aggressively, causing many unpleasant symptoms to the host (dEnfert and Hube, 2007; Tarlan and Rick, 2010; Burrus, 2012). *Candida* is able to produce toxins which cause severe long-term disruption of the immune system and may also attack the brain. (Foster, 2013; Borre, 2014).

The majority of the clinically used antifungals have various drawbacks in terms of toxicity, drug– drug interactions, lack of fungicidal efficacy, cost and emergence of resistant strains caused by the frequent use of some of them. In spite of the recent introduction of new antifungal drugs. Hence, there is a great demand for novel antifungal agents, justifying the intense search for new drugs that are more effective and less toxic than those already in use (Rapp, 2004; Kauffman, 2006).

Aromatic plants have been used in folk medicine as antimicrobial agents since ancient times (Grayer and Harborne, 1994; Cowan, 1999). The essential oils from many plants are known to possess antifungal activity (Dorman and Deans, 2000; Kalemba and Kunicka, 2003; Burt, 2004; Bakkali, *et al.*, 2008).

Essential oils have been empirically used as antimicrobial agents, but the spectrum of activity and mechanisms of action remain unknown for most of them. Although only limited consistent information exists about activity toward human fungal pathogens, some essential oils have shown important antifungal activity against yeasts (Pina-Vaz, *et al*., 2004; Cavaleiro, *et al*., 2006; El-Shouny,2006; Pinto, *et al*., 2006). The clove oil has been described as having useful antiseptic, analgesic and anesthetic effects (Chaieb, *et al.*, 2007a).

Previous studies have reported antifungal activity for clove oil (Velluti, *et al.,* 2004; Lo´ pez, *et al.*, 2005) and human pathogenic fungi (Gayoso, *et al.*, 2005; Chaieb, *et al.*, 2007b). Clove oil has also been tested as antifungal agents in animal models (Chami, *et al*., 2004a, b; Ahmad, *et al*., 2005).

The aim of this work is to evaluate yeast colonization among autistic children, explain the risk of yeast overgrowth in autistic behavior, and evaluate the antifungal activity of some plant extracts and some essential oils.

2. Material and Methods

2.1 Grouping of children

Children will be recruited among outpatients referred to the Clinical Genetics Department, National Research Centre. (All recruited patients signed informed consent according to the ethical committee of National Research Centre). This study included 25 children, diagnosed as autistic children (18 boys and 7 girls) and 10 normal children without any autistic features as control group (6 boys and 4 girls), their ages ranged from 3-9 years, the study was carried out between Marsh 2014 to August 2015.

Exclusion criteria include:

- Children under antifungal treatment.
- Children under cytotoxic or immunosuppressive drugs.
- Children with abnormal routine laboratory investigations; blood sugar, kidney function and liver function tests.

All children were subjected to

- Clinical Investigations and
- Stool culture for yeast growth.

2.2 Clinical investigation

Autistic children were subjected to meticulous and detailed history including personal, pregnancy, delivery, Prenatal and postnatal Pedigree construction with particular emphasis on consanguinity, other affected family members. Detailed Nutritional history, and GIT manifestation of children. Vaccination details, electroencephalography (EEG), complete audio logical evaluation, psychometric evaluation and rating of severity using Checklist. Autism Rating Scale (CARS), disorders were (CARS) Childhood autism rating scale, disorders were done as 30 serving as a cut off for a diagnosis of autism, mild autism (30- 36) moderate autism (37-45) and severe autism (46-60). (Eric, *et al*., 1988).

2.3 Stool culture for yeast growth

All children were subdivided into 3 subgroups according to stool culture result for yeast growth, which included heavy, moderate and no growth group.

2.3.1 Collection and culture of stool samples

Stool culture was obtained on Sabouraud dextrose agar (SDA) (SDA, Himedia, Mumbai, India) for isolation of yeast (Culture medium for fungal isolates), each 1litre of distilled water contain 20 g dextrose, 10 g peptone, 20 g agar, and 0.5 g from each chloramphenicol and cyclohexaimide were added to avoid bacterial contamination. (Larone, 1995).

Random stool samples were collected in sterile containers, immediate culture was done on (SDA) plate and incubated at 37^{o}C for 48 hours (negative growth when the stool samples yielded no colonies, heavy growth when samples yielded colonies all over the lines of plating out, low growth when samples yielded colonies more than negative growth and less than heavy growth.

2.3.2 Identification of samples

Gram stained smears were prepared using Gram stain from EDM Company and examined by ordinary light microscope for yeast cells, then all the yeast isolates were identified using API *Candida* (BioMérieux Vitek, Hazelwood, MO, USA) using the manual instructions and identified through specific number code 10 & 11.

2.4 Collection of plant materials and oils used in this study

The plants used in this study were obtained commercially, three plants were used. They were purchased from the local market of Tanta, Egypt. Botanical identification of the plants samples was carried out in Botany Department, Faculty of Science, Tanta University. The oils used in this study also were obtained commercially, three oils were used.

2.4.1 Preparation of plant extracts

Dried plants were cut into small pieces using a sharp knife. They were converted into powder using a blender. 20 g of each plant powder were taken for extraction. The solvents extraction was done by using acetone and methanol (200 ml) separately for 48 hrs. at room temperature. And filtered using Whatman˝s No1filter paper (9 cm). The filtrate obtained was concentrated at 35°C in a rotary evaporator to obtain the crude extract. The crude extracts were kept at 4°C until further uses (Djeussi, *et al.,* 2013).

2.4.2 Preparation of oils

Oils were used as a crude (undiluted) oil and also as diluted oil by DMSO (Dimethyl sulphoxide) in a dilution of 1:1 and 1:2. Mixture between oils made by 1:1 (v/v) with Addison of tween 80.

2.5 Screening for antifungal activity of plant extracts and oils

The five isolates were screened for their susceptibility to different plants extracts and oils using the agar well diffusion method to determine the inhibition zone diameter, that oil and crude plant extracts were made into suspensions using DMSO, the concentration of suspensions was made 100 mg/ml from each plant extract, and in a dilution of 1:1, 1:2, for each oil.

Each yeast isolate was sub cultured overnight in Sabouraud dextrose broth, then adjusted to obtain turbidity equal to 10^6 CFU/ml using the turbid meter, 100 µl of each broth cultures was inoculated into three well dried plates of Sabouraud dextrose agar (replicate) and was spread homogeneously using sterile glass rod and left to dry for 15 min. Wells of 8 mm diameter were made in nutrient agar surface using sterile borer. A volume (50 µl) of each extract suspension was inserted into the wells by automatic pipette, the plates were incubated at 37°C for 24 hrs. after incubation time the inhibition zones diameters in mm were measured, these inhibition zones were compared with negative control (50 µl DMSO) and a positive control (Nystatin).

2.6 Determination of minimum inhibitory concentration (MIC)

The best extracts that showed antifungal activity were chosen and later tested to determine the minimum inhibitory concentration (MIC) by using the agar diffusion method using suspension of plants in concentration of 12.5, 25, 50, 100 and 200 mg/ml.

3. Statistical Analysis

The data were analyzed using Statistical Program for Social Studies (SPSS version 20).

4. Results and Discussion

The demographic characteristics of autistic children (test group) are summarized in table 1 showed yeast growth and autism severity among 25 tested children (included 18 males (72%) and 7 females (28%), with mean age 5.02±1.9 years). Results revealed severity of autism depending on childhood autism rating scale (CARS) test. CARS test disorders were done as 30 serving as a cut off for a diagnosis of autism, mild autism (30-36) moderate autism (37-45) and severe autism (46-60)**,** and concerning the yeast growth in CFU/ml.

The demographic characteristics of normal children (control group) are summarized in table 2 the control group (included 10 children 6 males (60%) and 4 females (40%), with mean age 5.9±1.9 years). Results revealed no autism features among these children according to CARS test. Stool culture on Sabouraud dextrose agar (SDA) was done for both groups for yeast growth in CFU/ml. Yeast growth in autistic and normal children are summarized in table 3 showed 19 (76%) cases were positive for yeast growth and 6 (24%) cases were negative growth in study group. Four cases (40%) were positive for yeast growth from the control group. These results were in agreement with Emam, *et al*. (2012) who reported that there was increased rate of infection by yeast in autism (81.9%) versus control group (28%). Horvath, (1999) also showed that there was increased rate of positive fungal culture for yeast in the duodenal juice (43%) of children with autism undergoing endoscopies more than had the age matched controls with other gastrointestinal problems requiring endoscopies (23%). The yeast growth was classified in to three levels high, low and nil, cases with negative growth of yeast were increased in control group when compared with autistic group, while there was increase in cases of heavy growth of yeast in autistic group compared with the control group. This finding had a similarity with the data recorded by Campbell, (1983) who reported that autism was associated with GIT infection with *Candida albicans;* a sign of impaired immune functions resulting in the overgrowth of yeast in the body. A complete agreement was noticed with the study of Emam, *et al*. (2012) who found that there was increase of heavy growth of yeast in autistic group compared with the control group. In contrast to our findings, Adams, *et al*. (2011) stated that yeast was rarely observed in the stool cultures of the autism and control groups.

Table 1: Demographic characteristic of the autistic children.

Autism group of children						
Child No.	Age		Sex.	CARS Score	Autism severity	Yeast growth CFU/ml
	Year	Month				
1	3	11	M	44	Moderate	$2x10^7$
2	3	6	M	49.5	Severe	$2x10^7$
3	3	5	M	48	Severe	$2x10^3$
4	3	0	M	34.5	Mild	$6x10^2$
5	8	9	M	33	Mild	$2x10^7$
6	3	7	F	38.5	Moderate	0
7	3	0	F	39.5	Moderate	$2x10^7$
8	5	6	F	40	Moderate	0
9	4	0	M	48	Severe	$2x10^3$
10	7	0	M	46.5	Severe	$2x10^6$
11	3	0	F	46	Severe	$1x10^7$
12	3	3	F	47	Severe	$38x10^5$
13	3	2	M	43.9	Moderate	$18x10^3$
14	6	0	M	43	Moderate	$2x10^3$
15	5	0	F	47	Severe	$2x10^3$

16	8	7	M	37.5	Moderate	0
17	6	0	M	34	Mild	20
18v	5	0	M	52.5	Severe	0
19	6	0	M	46.7	Severe	$2x10^7$
20	5	5	M	37	Moderate	$2x10^5$
21	3	0	M	44.5	Moderate	$26x10^7$
22	5	0	M	32	Mild	$12x10^7$
23	4	5	M	47.4	Severe	0
24	7	0	F	48	Severe	$2x10^5$
25	8	9	M	34	Mild	0

Where (**M)** Male, (**F**) female. **(CARS)** Childhood autism rating scale, disorders were done as 30 serving as a cut off for a diagnosis of autism, mild autism (30- 36) moderate autism (37-45) and severe autism (46-60).

Table 2: Demographic characteristic of the normal children (Control group).

Control group of children					
Child No.	**Age**		**Sex**	**CARS score**	**Yeast growth CFU/ml**
	Year	**Month**			
1	3	5	M	< 30	0
2	5	0	M	< 30	$4x10^3$
3	6	2	M	< 30	0
4	4	6	F	< 30	$2x10^2$
5	3	0	F	< 30	0
6	8	0	M	< 30	$2x10^7$
7	7	5	M	< 30	0
8	5	8	F	< 30	0
9	6	5	M	< 30	$3x10^3$
10	9	0	F	< 30	0

Where **(M)** Male**, (F)** female**. (CARS)** Childhood autism rating scale, disorders were done as 30 serving as a cut off for a diagnosis of autism, mild autism (30- 36) moderate autism (37-45) and severe autism (46-60).

Table 3. Yeast growth in autistic and normal (control) children.

Yeast growth	**Groups**			
	Autistic children (n = 25)		**Normal children (n = 10)**	
	No.	**%**	**No.**	**%**
High	12	48	1	10
Low	7	28	3	30
Nil	6	24	6	60
Total growth	19	76	4	40

High growth when number of colonies (CFU/ml) $\geq 1x10^6$, low growth when number of colonies (CFU/ml) $< 1x10^6$, Nil growth when no yeast growth.

Characteristics of the autistic group regarding sex and severity of autism were summarized in table 4. The results revealed that 7 cases of males versus 4 cases of females have sever autism with percentage 38.9% - 57.1%, respectively during the present survey. In this study, it could not raise up a clear satisfactory relationship between gender and autism severity. This might be due to the few number of the herein investigated cases of autistic children. Meng-Chuan, *et al*. (2014) said that autism severity stimulated by the male bias in autism prevalence. Findings are complex and do not always relate to each other in a straightforward manner. but interlinked questions on the relationship between sex/gender differences and autism remain under addressed and also the neuroanatomical differences between male and female children with autism spectrum disorders (ASD) are an intriguing and still poorly investigated issue. On the other hand, other studies reported no significant gender differences in the symptom profile of preschoolers with ASD or gender differences that reflect those found in typical young children. In a similar vein, a systematic review and meta-analysis of the impact of age and gender on the core autistic symptoms described no differences in symptom severity between toddler/preschooler males and females with ASD (Zwaigenbaum, *et al*., 2012; Andersson, *et al*., 2013; van Wijngaarden-Cremers, *et al*., 2013).

Table 4: Relation between sex and autism severity.

Autism severity	**Autism group**			
	Male		**Female**	
	No.	**%**	**No.**	**%**
Sever	7	38.9	4	57.1
Moderate	6	33.3	3	42.9
Mild	5	27.8	0	0
Total	18	100	7	100

Table 5 revealed the relation between yeast growth in stool cultures and the severity of autism. The data showed that sever autistic children had heavy yeast growth with percentage 45.4%, low growth with percentage 36.4% and absence of yeast growth with percentage 18.2%. Moderate severity recorded equal percentage for heavy, low and no yeast growth with percentage 33.3%. Mild severity showed heavy growth with percentage 60% and equal percentage of low and absence of yeast growth with percentage 20%. This indicated that heavy growth of yeast among autistic children is a features common without considering the level of autism severity. Emam, (2012) reported that number of patients with negative stool culture growth was significantly increased (P = 0.027) in mild-moderate group compared with severe group; while there was statistically insignificant difference in number of minimal and heavy yeast growth.

Table 5: Correlation between Yeast growth in stool culture and autism Severity.

Yeast growth	Autism severity						Total growth	
	Severe n =11		Moderate n = 9		Mild n = 5			
	No.	%	No.	%	No.	%	No.	%
High	5	45.4	3	33.3	3	60	11	44
Low	4	36.4	3	33.3	1	20	8	32
Nil	2	18.2	3	33.3	1	20	6	24

Where n is total number of cases according to CARS.

High growth when number of colonies (CFU/ml) ≥ $1x10^6$, low growth when number of colonies (CFU/ml) < $1x10^6$, and nil when no yeast growth.

Incidence of yeast isolates among autistic children and its effect on autism severity were summarized in table 6. The most common yeast isolated from autistic children during the present survey was *Candida krusei*. It was positively isolated from 8 cases with percentage 42.1% followed by *Candida albicans* which was isolated from 4 cases with percentage 21.1%, followed by *Tricosporon* mucoides. that was isolated from 3 cases with percentage 15.8%. *Candida dublinesis* and *Candida glabarata* recorded low incidence with occurrence percentage 10.5%. Concerning the effectiveness of yeast infection on the autism severity, the data showed that the most isolated yeast causing high severity of autism was *Candida krusei* which present among sever cases with percentage of 54.5%. According to Emam, *et al.* (2012) survey, *Candida albicans* may be etiological factor lead to excessive ammonia in gut which is responsible of autistic behavior in children. A disagreement, their mentioned data revealed that *Candida albicans* was the most common. The herein obtained results were mostly agreed with Colombo, (2011) who stated that recorded yeast in their reported autism cases, the non-*albicans Candida* species, *C. krusei, C. tropicalis* and non-*Candida* strains including *T. mucoides* and *S. cerevisiae* were determined among yeasts deposited in the last 3 years. Furthermore, these investigations mentioned that *Trichosporon* species may be a part of human intestinal flora.

Table 6: Incidence of yeast isolates and its effect on severity of autism among autistic children.

Yeast isolates	Incidence of yeast isolates		Autism sever cases	
	No. of isolates	Out of total (%)	No.	%
Candida albicans	4	21.1	1	9.1
Candida dublinesis	2	10.5	1	9.1
Candida glabarata	2	10.5	1	9.1
Candida krusei	8	42.1	6	54.5
Tricosporon mucoides	3	15.8	2	18.2
Total	19	100	11	100

Where No. is total number of sever autism children according to CARS.

Antifungal activity of thyme, clove and chamomile extracts against isolated yeasts from autistic children are summarized in table 7, all of them gave high significant and acetone extract of clove resulted in the highest inhibition effect on isolated yeasts with total mean of inhibition 25.7±1.5, followed by chamomile then thyme extractes. According to Kaoutar, *et al.* (2010), extract of clove was the most effective as an antibacterial agent. Mau, *et al.*, (2001) and De, *et al* (1999) studies. confirmed the inhibitory effect of cinnamon on few species of *Candida*. Also, in a study on 12 plant species with therapeutic effect, cinnamon, spearmints, and thyme had more antifungal activity (Soliman and Badeaa, 2002). Thyme showed moderate antifungal effect (Suhr and Nielsen, 2003). In another study, the inhibitory effect of cinnamon, thyme and clove against a few different fungi such as *Candida albicans* was cleared Suhr and Nielsen ,2003. The antifungal effect of chamomile was observed by (McKay and Blumberg, 2006 and Cervenka, *et al.* 2006). Recently, El-Shouny *et al.* (2014) recorded high antifungal activity of chamomile acetone extract against *Candida albicans* (18mm) at a concentration of 400 µg/ml.

MIC of clove acetone extract was done and summarized in table 8 which revealed that 25 µg/ml was the lowest concentration that could inhibit growth of isolated yeasts from autistic children, except with *Candida dublinesi*, MIC was 100 µg/ml. This indicated that clove exhibited the strongest antifungal activity against isolated yeasts, but *Candida dublinesis* needs high concentration of clove to inhibit its growth. The concentrations 25,50 and 100 µg / ml gave highly significant anti-candidal effects.

Table 7: Antifungal activity of different plant extracts against yeasts isolated from autistic children.

Yeast isolates	Thyme extract (100 µg / ml)		Clove extract (100 µg / ml)		Chamomile extract (100 µg / ml)		Nystatin
	Ethanol	Acetone	Ethanol	Acetone	Ethanol	Acetone	
	Zone of inhibition (mm)						
Candida albicans	11± 0.0	12±0.0	23±1.0	27±0.6	13±0.6	14±1.0	16±1.0
Candida dublinesis	0± 0.0	0±0.0	20±1.5	25±0.0	14±1.0	15±0.6	17±1.0
Candida glabarata	0± 0.0	12±0.6	22±1.0	25±1.0	11±1.0	12±1.0	15±1.0
Candida krusei	11± 0.6	13±0.9	16±0.5	24±0.0	12±1.0	15±1.0	15±1.0
Tricosporon mucoides	0± 0.0	12±1.5	21±2.0	27±1.0	10±0.0	14±1.0	13±1.0
Total mean	4.5±5.8	10.0±5.2	20.6±2.5	25.7±1.5	12.1±1.7	14.13±1.5	15.2±1.6
p. value	0.000***	0.000***	0.002***	0.000***	0.001***	0.007**	0.007**

Total mean values are mean inhibition zone (mm) ± S.D of three replicates.
insignificant (ns) difference at p >0.05, significant (*) at P ≤ 0.05, highly significant (**) at P ≤0.01 and very highly significant (***) at P ≤ 0.001.

Table 8: MIC of acetone extract of Clove against yeast isolated from autistic children.

Yeast isolates	Conc. of acetone clove extract (µg/ml)				
	12.5	25	50	100	200
	Zone of inhibition (mm)				
Candida albicans	0± 0.0	10±1.0	20±1.0	27±0.6	29±1.0
Candida dublinesis	0± 0.0	0.0±0.0	0.0±0.0	25±0.0	27±1.0
Candida glabarata	0± 0.0	16±1.00	23±1.0	25±1.0	26±1.0
Candida krusei	0± 0.0	16±1.0	22±1.0	24±0.0	28±1.0
Tricosporon mucoides	0± 0.0	17±1.0	20±1.0	27±1.0	28±1.0
Total mean	0± 0.0	11±6.7	17±8.9	25.7±1.5	27±1.3
p. value	-	.000***	.000***	0.000***	.037**

(MIC) minimum inhibition concentration. Total mean values are mean inhibition zone (mm) ± S.D of three replicates.
insignificant (ns) difference at p >0.05, significant (*) at P ≤ 0.05, highly significant (**) at P ≤0.01 and very highly significant (***) at P ≤ 0.001.

Antifungal activity of clove, cinnamon and rosemary essential oils with different dilution against isolated yeast from autistic children are summarized in table 9. The best inhibitory effect was recorded by clove oil was in an undiluted form than in diluted one with total mean of inhibition zone (25±9.4) and cinnamon oil in diluted form 1:2 with total mean inhibition zone (16±10.4). No clear increase antifungal effect was observed by mixing both of them. It is clearly noticed that all oils in undiluted form gave high statistically significant.

The obtained data showed that, clove oil recorded a broad-spectrum inhibition against the isolated yeasts. Ahmad, *et al.* (2005) reported that clove oil possess strong antifungal activity against *C. albicans* and Baratta *et al*. (1998) found that cinnamon and clove oils have been reported by many researchers as a good source of antifungal compounds.

This is in agreement with Pinto, *et al.* (2009) who found that clove oil inhibited growth not only dermatophytes, *Aspergillus* and *Candida* species such as (*C. albicans*, *C. tropicalis* and *C. parapsilosis*), but also fluconazole-resistant *C. albicans* isolates, *C. krusei*, which was intrinsically resistant to fluconazole, and *C. glabrata*, whose resistance is easily inducible. Clove extract could play a leading role for the inhibitory effect of *Candida* growth. *In vitro* the inhibitory effect of clove oil according to our survey was directly related to its concentration. Palhano *et al*. (2004) found that the inhibitory effects were greater at increasing oil concentrations. El-Shouny (2006) reported that thyme oil was the most effective antifungal agent, followed by cinnamon and clove oils against *Candida albicans* and four filamentous fungi.

Some investigators reported the antifungal effectiveness of rosemary oil (Hammer, *et al*., 1999; Ahmad and Beg, 2001; Bozin, *et al*., 2007). On conterarry, Pozzatti, *et al.* (2008) stated the inefficiency of rosemary oil against *Candida* isolates.

Table 9: Antifungal activity of essential oils against yeasts isolated from autistic children.

Yeast isolates	Clove oil*			Cinnamon oil**			Rosemary oil***			Mixture of the * and**	Nystatin
	Undiluted oil	Diluted oil 1:1	Diluted oil 1:2	Undiluted oil	Diluted oil 1:1	Diluted oil 1:2	Undiluted oil	Diluted oil 1:1	Diluted oil 1:2		
	Zone of inhibition (mm)										
Candida albicans	17±1.0	20±1.0	24±1.0	12±1.0	18±0.0	29±1.0	0.0±0.0	0.0±0.0	0.0±0.0	17±1.0	16±1.0
Candida dublinesis	25±1.0	21±1.0	18±1.0	0.0±0.0	0.0±0.0	25±1.0	0.0±0.0	0.0±0.0	0.0±0.0	20±1.0	17±1.0
Candida glabarata	42±0.0	30±0.0	24±1.0	10±1.0	12±1.0	15±1.0	0.0±0.0	0.0±0.0	0.0±0.0	16±1.0	15±1.0
Candida Krusei	17±1.0	0.0±0.0	20±0.0	11±1.0	12±1.0	14±1.0	11±1.0	0.0±0.0	0.0±0.0	18±1.0	15±1.0
Tricosporon mucoides	24±1.0	0.0±0.0	27±1.0	21±1.0	0.0±0.0	0.0±0.0	6±1.0	0.0±0.0	0.0±0.0	0.0±0.0	13±1.0
Total mean	25±9.4	14±12.5	22±3.4	10±6.9	8±7.4	16±10.4	8.5±4.6	0.0±0.0	0.0±0.0	14.2±7.5	15.2±1.6
P. value	0.000***	0.000***	0.00**	0.00**	0.00***	0.00***	0.00***	-	-	0.000***	0.007**

Total mean values are mean inhibition zone (mm) ± S.D of three replicates.
In significant (ns) difference at p >0.05, significant (*) at P ≤ 0.05, highly significant (**) at P ≤0.01 and very highly significant (***) at P ≤ 0.001.

5. Conclusion

Yeast infection may be a part of syndrome related to the immune system and autism severity. The obtained data indicated that the clove extract, clove oil and cinnamon oil have interesting potential as a therapeutic option against yeasts that are pathogenic to autistic children.

6. Recommendation

The exact mechanism by which yeasts affect autistic children needs further research. Confirmatory investigations of the safety of herbal medications for autistic children are also of great importance.

7. Acknowledgment

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