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Experimental and DFT calculation studies on boric acid and salicylic acid-boric acid-ethanol solution

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ABSTRACT

In the present study, the vibrational analysis of boric acid molecule and boric acid solution prepared as a magistral ear drug was experimentally performed and its chemical characteristics was examined for the first time using FT-IR and NMR chemical shift spectra. The boric acid and salicylic acid powders were analyzed by X-ray powder diffraction. The particle size of boric acid and salicylic acid were determined as 457 µm in Dv(50) and 59.8 µm in Dv(50) respectively using particle size analysis device. The optimized molecular structure, vibrational wavenumbers and the highest and the lowest occupied molecular orbital analyses of boric acid molecule were theoretically obtained with DFT method at the B3LYP/6-311++G(d,p) basis set. The vibrational wavenumbers were found to be consistent with the literature results and the experimental data obtained in the current work. Boric acid solution was found to exhibit antimicrobial activity after the agar disk diffusion and agar well diffusion tests performed on *Candida albicans* (ATCC 10231) strain.

1. Introduction

As a hydrate of boron trioxide, boric acid is available in tri-hydrate or monohydrate form and it has the chemical formula B_2O_3-$3H_20$, H_3BO_3 or $B(OH)_3$. It is also termed as orthoboric acid, boracic acid, hydrogen borate, borofax and acidum boricum. As also indicated by the formula "H_3BO_3" boric acid is in fact a tribasic bronsted acid as in the case of phosphoric acid (H_3PO_4). Its monohydrate form is termed as metaboric acid and represented with B_2O_3-H_2O or HBO_2 [1]. Boric acid has high solubility in aquatic media and various organic solvents such as normal alcohols, and poly or polyhydric alcohols. Boric acid yields stable complexes by reactions with pyridine, dioxane or dialcohols. Solubility of this molecule increases depending on temperature in aquatic environment [2]. Bezerra da Silva et al, [3] evaluated the structural, electronic and optical properties of bulk boric acid 2A and 3T polymorphs using density functional theory at the local density (LDA) and generalized gradient calculation (GGA) levels. In agriculture and forestry, boric acid is used as a pesticide to kill mites, fungi, plants and insects including fleas, termites, cockroaches and wood decay fungi. Other applications of boric acid include food preservative, newborn baby's nurseries and antiseptic use [4]. With its odorless, crystal white solid form, boric acid is used in ceramics and cosmetics industries in the production of detergents, borosilicate glasses, textile products, fiberglass and other chemical applications. The boiling point of boric acid is 1860°C and its pH is 6.1 at 20°C (in 0.1 % solution), 5.1 (in 1.0 % solution) and 3.7 (in 4.7 % solution). Its solubility in water is 4.7 % in 20°C and 27.5 % in 100°C [5].

The present study aims to examine experimentally the vibrational FT-IR and ^{1}H NMR isotropic chemical shift spectroscopic properties of a magistral ear drug with antiseptic and antifungal properties prepared using boric acid, salicylic acid and ethanol. The antimicrobial activity of boric acid solution was experimentally verified on a culture of *C. albicans* –ATCC 10231 strain.

2. Materials and methods

2.1. Materials and apparatus

Boric acid was purchased from Suvar Kimya in powder form. The chemical formula of boric acid is H_3BO_3. The CAS number and EC number is 10043-35-3 and 233-139-2, respectively. The molecular weight is 61.83 g/mol and the melting point is at 185 ^{0}C. Its relative density is 1.44 g/cm^3 at 20 ^{0}C and water solubility is 50 g/l at 21 ^{0}C. Boric acid has 3.8-4.8 pH in 33 g/l at 20 ^{0}C and its vapor pressure is 2.7 hPa at 20 ^{0}C. The partition coefficient (n-octanol/water) is 0.757 at 25 ^{0}C (logPOW).

Salicylic acid was purchased from Galenik Medicines

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and Chemical Materials Warehouse. The chemical formula of salicylic acid is HOC_6H_4COOH or $C_7H_6O_3$. The CAS number and EC number is 69-72-7 and 200-712-3, respectively. The molecular weight is 138.12 g/mol and the melting point is in the range of 157 ^{0}C - 159 ^{0}C. Its relative density is 1.443 g/cm^3 at 20 ^{0}C and water solubility is 2 g/l at 20 ^{0}C. Salicylic acid has 2.4 pH value in saturated solution and its vapor pressure is <1 hPa at 100 ^{0}C. Its partition coefficient (n-octanol/water) is 2.26 (logPOW).

Ethanol was purchased from Merko Kimya. Its alcohol content is 96.5 %v/v and density is 0.8052 g/ml at 20 ^{0}C in vacuum.

The FT-IR spectrum of boric acid molecule was recorded at room temperature in the region 400-4000 cm^{-1}, using potassium bromide (KBr) pellet, on Fourier Transform Infrared spectrometer in solid phase of the sample. The ^{1}H NMR chemical shift spectra of boric acid and the salicylic acid-boric acid-ethanol solution were recorded using Premium Compact NMR device at 600 MHz frequency and 14.1 Tesla field power. The used solvent was methanol-d_4.

2.2. Preparation

Magistral prescriptions are patient-specific prescriptions given by physicians. The specimens used in the present study comprise of boric acid solution. This solution was prepared using boric acid, salicylic acid and ethanol. The solution, which has antifungal property, is a mixture of 3 g salicylic acid ($C_7H_6O_3$/HOC_6H_4COOH), 6 g boric acid (H_3BO_3) and 96% ethanol (C_2H_6O) mixed at 40 ^{0}C in 100 mL. The pH value of salicylic acid-boric acid-ethanol solutions was found as pH 6, using Litmus test. Salicylic acid concentration was 161 mg/L and boric acid concentration was 321 mg/L in the salicylic acid-boric acid-ethanol solution at 24 ^{0}C.

3. Results and discussions

3.1. Geometry and analysis of molecular structure

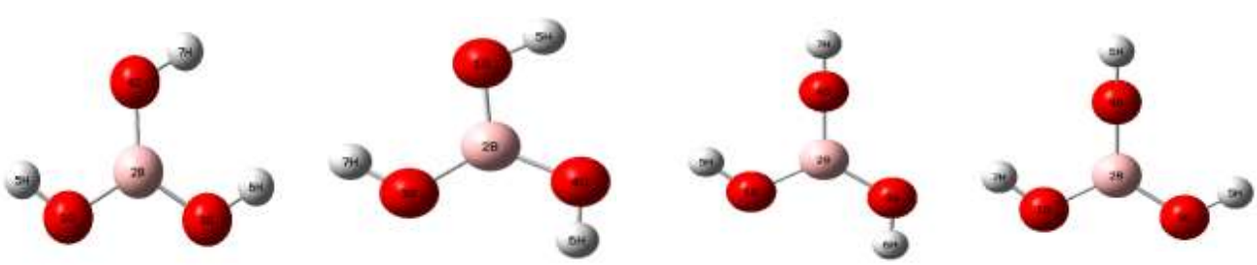
The central boron atom on boric acid molecule is connected to three hydroxyl (-OH) groups consisting of strong hydrogen bonding as given in Figure 1. The structure of orthoboric acid, $B(OH)_3$, was initially introduced by Zachariasen in 1934 [6] and all intensity measurements were made with a modified XRD-3 spectrometer using Cu Ks radiation [6]. The triclinic unit cell, containing four molecules $B(OH)_3$, has dimensions a_1 = 7.039 Å, a_2=7.053 Å, a_3= 6.578 Å, α_1= 92.58^0, $\alpha_{2=}$101.17^0, $\alpha_{3=}$119.83°. The bond distances in a $B(OH)_3$ molecule are B-O = 1.361 Å, O-H---- 0.88 Å, with a value of 114^0 for the oxygen bond angle. The O-H…O distance between the molecules is 2.720 Å [7]. Boric acid also known as orthoboric acid is a white crystalline, oxygen-bearing acid of boron. The most stable conformation of boric acid has C_{3h} symmetry (1-C_{3h}) [8]. Boric acid, salicylic acid and ethanol solution was prepared as a magistral drug. Boric acid and salicylic acid were pulverized and mixed in a mortar and dissolved homogeneously with addition of ethanol [9].

At B3LYP/6-311++G(d,p) basis set, O(3)-H(7), O(4)-H(6) and O(1)-H(5) bond lengths were respectively computed as 0.96179 Å, 0.96172 Å and 0.96174 Å. B(2)-O(1), B(2)-O(4) and B(2)-O(3) bond lengths were respectively computed as 1.36964 Å, 1.36989 Å and 1.36980 Å. O(1)–B(2)–O(4), O(3)–B(2)–O(4) and O(1)–B(2)–O(3) bond angles were respectively calculated as 119.99438^0, 119.99317^0 and 120.01244^0 as shown in Figure 1. B(2)-O(1)-H(5), B(2)-O(3)-H(7) and B(2)-O(4)-H(6) bond angles were calculated as 112.73745^0, 112.78165^0 and 112.74555^0, respectively.

Figure 2. a) gives the optimized molecule structure of boric acid molecule. In the B3LYP/6-311++ G(d,p) level, the calculated HOMO and LUMO energy values are -9.1325 eV and -0.5763 eV, respectively, as given in Figure 2 (b) and (c). The calculated molecular energy and dipole moment values of the boric acid molecule are -252.58568942 a.u. and 0.0107 Debye, respectively.

3.2. Computational procedures

The optimized molecular geometry, vibrational wavenumbers, NMR chemical shifts of the boric acid molecule were computed using DFT/B3LYP (Becke's three parameter exact exchange-functional (B3) combined with gradient-corrected correlational functional of Lee, Yang, Parr (LYP) method with 6-311++G(d,p), basis set [10,11]. Vibrational wavenumbers, geometric parameters and other molecular properties were



a) **b)** **c)** **d)**

Figure 1. The conformers of boric acid **a)** 1-Cs **b)** 1-C3h **c)** 1-TS1 **d)** 1-TS2 (Cs) [8]

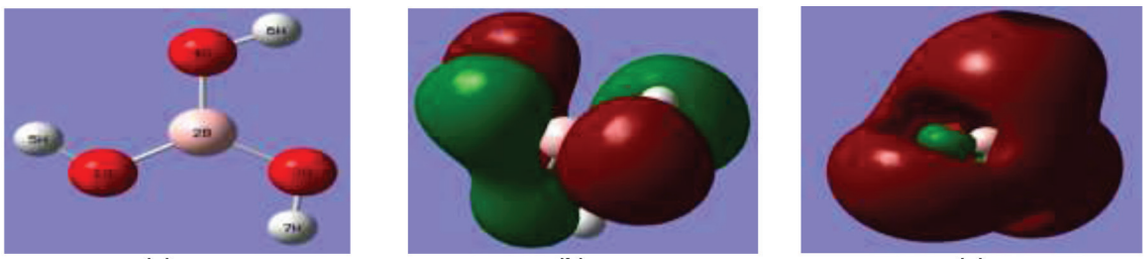


Figure 2. a) The optimized molecular structure, **b)** E(HOMO) = -9.1325 eV and **c)** E(LUMO) = -0.5763 eV energies with the B3LYP/6-311++G(d,p) level

performed using Gauss View molecular visualization program and Gaussian 09W software on a computer system. ^{1}H NMR chemical shift calculated with gauge-including atomic orbital (GIAO) approach using Gaussview software shows good agreement with experimental chemical shift [12-14]. The calculations were theoretically performed with methanol-d_4 and in vacuum. Also, scaling factors were used between theoretical and experimental vibrational wavenumbers. The computed vibrational wavenumbers were scaled with 0.983 for frequencies less than 1700 cm^{-1} and 0.958 for frequencies higher than 1700 cm^{-1} at B3LYP/6-311++G(d,p) basis set [15].

3.3. Vibrational frequency analysis

The vibration frequencies of FT-IR were experimentally obtained at 3392.19 cm^{-1}, 2849.98 cm^{-1}, 2517.20 cm^{-1}, 2386.02 cm^{-1}, 2262.42 cm-1, 1436.55 cm^{-1}, 1192.39 cm^{-1}, 883.26 cm^{-1}, 723.69 cm^{-1}, 635.13 cm^{-1} and 544.39 cm^{-1} as given in Figure 3. 3226 cm^{-1}, 2507 cm^{-1}, 2679 cm^{-1}, 2262 cm^{-1}, 1480 cm^{-1}, 1198 cm^{-1}, 885 cm^{-1}, 747 cm^{-1}, 675 cm^{-1}, 648 cm^{-1} and 649 cm^{-1} frequencies values of boric acid (HIT-NO=7295 and SDBS-NO=15119) are experimentally given in Ref [16]. The O-H stretching vibrations of boric acid appear at 3413 and 3788 cm^{-1}, at 3475 and 3781 cm^{-1}, and at 3778 and 3782 cm^{-1} in reactant, transition state and product system, respectively [17]. The O-H stretching vibrations of boric acid experimentally appear at 3392.19 cm^{-1} in this study. IR active peak (1500-1300 cm^{-1}) for the asymmetric B-O stretching (v_3) as well as an inactive or weakly IR active symmetric B-O stretching (v_1) band (1100-950 cm^{-1}) are explained and there are additional broad peaks that arise from in plane B-O-H bending (1300-1000 cm^{-1}) and out of plane bending (850-700 cm^{-1}) [18]. These values are in a good agreement with the recorded and computed data in our study. The calculated FT-IR and Raman analysis of boric acid powder with B3LYP/6-311++ G(d,p) was given in Figure 4. The experimental and calculated vibrational frequencies and their assignments of boric acid are given in Table 1.

The particle size spectrum of boric acid and salicylic acid were recorded using particle size analysis device with Malvern, Mastersizer 3000 as given in Figure 5. The boric acid refractive index is 1.470 and dispersant refractive index of water is 1.330. The specific surface area was 16.68 m^2/kg. The uniformity of concentration

Table 1. The experimental and calculated vibrational frequencies and their assignments of boric acid

Assignments	Experimental IR (cm^{-1})				Calculated IR (cm^{-1}) B3LYP/6-311++G(d,p) level			
	Freq.	% T	[a]Freq.	% T	Unscaled freq.	Scaled freq.	IR int.	SRaman act.
v_sOH (12) + v_sOH (17) + v_sOH (71)	3392.19	36.82	3213	4	3872.69	3710.04	9.3203	153.5317
v_sOH (55) + v_sOH (33)					3871.95	3709.33	104.0412	44.6803
v_sOH (18) + v_sOH (65) + v_sOH (27)					3871.72	3709.11	112.6234	34.8806
δ_sHOB (13) + vBO (31)+ vBO (44)	1436.55	36.79	1461	6	1438.64	1414.18	458.3690	0.1775
δ_sHOB (10) + vBO (35)+ vBO (40)					1438.20	1413.75	457.6189	0.1784
δHBO (92)	1192.39	39.02	1194	10	1027.92	1010.45	1.4852	0.3253
δ_sHBO (13)+ δ_sHBO (61)+ vBO (16)					1018.81	1001.49	172.1715	2.8035
δ_sHBO (18) + δ_sHBO (58) + vBO (10) + vBO (12)					1017.71	1000.41	171.2860	2.7965
v_sBO (95)			878	70	872.87	858.03	0.0002	9.6802
γOOOB(99)			676	77	672.82	661.38	103.0060	0.0001
τHOBO (26) + τHOBO (73)	544.39	41.32			534.42	525.33	0.0806	0.5338
τHOBO (98)					438.48	431.03	356.6992	0.0009
δOBO (32) + δOBO (58)					426.02	418.78	30.7545	0.5673
δOBO (31) + δOBO (57)					425.45	418.22	30.8943	0.5647

ν, stretching; δ, in-plane bending; δ_s, scissoring and symmetric bending; w, wagging; ρ, rocking; I_{IR}, IR intensity; S_{Raman}, Raman scattering activity; s, symmetric; as, asymmetric; PED, potential energy distribution.

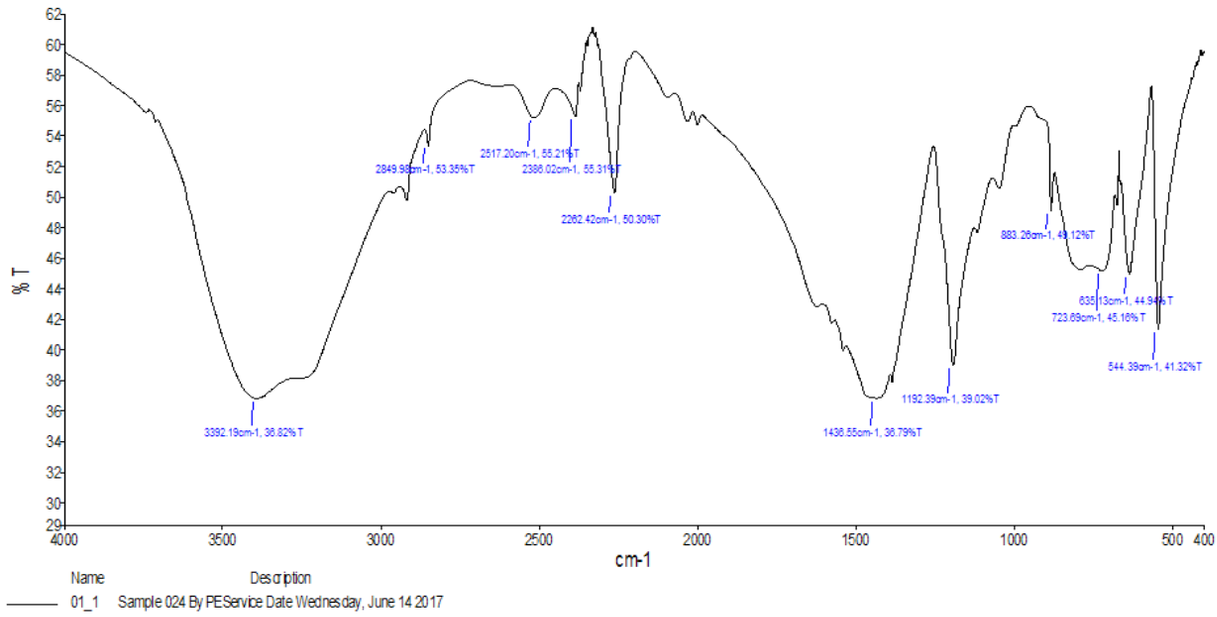


Figure 3. Experimental FT-IR analysis of boric acid powder

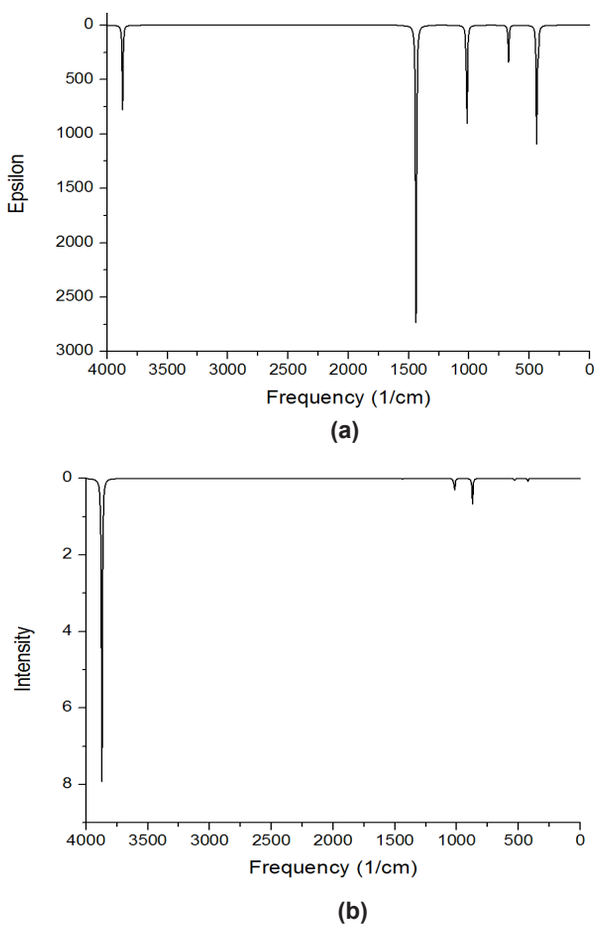


Figure 4. The calculated FT-IR and Raman analysis of boric acid powder with B3LYP/6-311++ G(d,p)

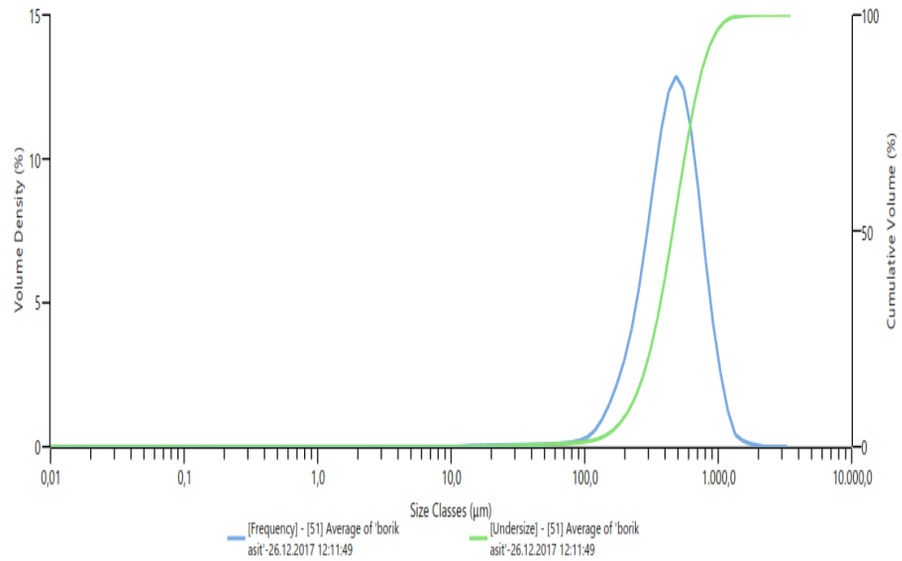
is 0.394. The results were obtained as 226 μm in Dv (10), 457 μm in Dv(50) and 805 μm in Dv (90). The salicylic acid refractive index is 1.537 and dispersant refractive index of water is 1.330. The specific surface area was 210.4 m^2/kg. The uniformity of concentration is 0.598. The results were obtained 17.0 μm in Dv (10), 59.8 μm in Dv(50) and 134 μm in Dv (90).

The boric acid was analyzed by X-ray powder diffraction using X-ray diffractometer device, RIGAKU, SmartLab model. X-ray powder diffraction analysis of boric acid and salicylic acid are shown in Figure 6. XRD patterns of the hexagonal structure of boric acid (HBA) and triclinic boric acid (TBA) were given as in 10^0-65^0. 2θ range [19]. XRD patterns of boric acid produced from a borax solution with a concentration of 1180 kg/m^3 was given in Ref [20] and as-prepared powder was crystalized as single-phase boric acid [20]. Accordingly XRD analysis was performed to verify the purity of the materials, and the results were in agreement with those reported in the literature.

3.4. ^{1}H chemical shift analyses

The NMR chemical shift spectra of boric acid and the salicylic acid-boric acid-ethanol solution were recorded at 600 MHz frequency and 14.1 Tesla field power. The used solvent was experimentally methanol-d_4. The chemical shift at ^{1}H NMR was generally found in the range of 1-10 ppm. The experimental ^{1}H NMR chemical shift spectra of boric acid and salicylic acid-boric acid-ethanol solution are given in Figure 7 and Figure 8. ^{1}H chemical shifts of boric acid are experimentally measured in the range of 0.871- 7.44 ppm. ^{1}H NMR chemical shifts of three hydrogen atoms were obtained at 3.297 ppm, 3.178 ppm and 3.176 ppm, respectively. The calculated NMR chemical shifts of hydrogen atoms (H6, H5 and H7) were 3.2921 ppm, 3.2887 ppm, 3.2886 ppm in methanol and 3.3032 ppm, 3.2993 ppm and 3.2943 ppm in vacuum, respectively.

a) Boric acid



b) Salicylic acid

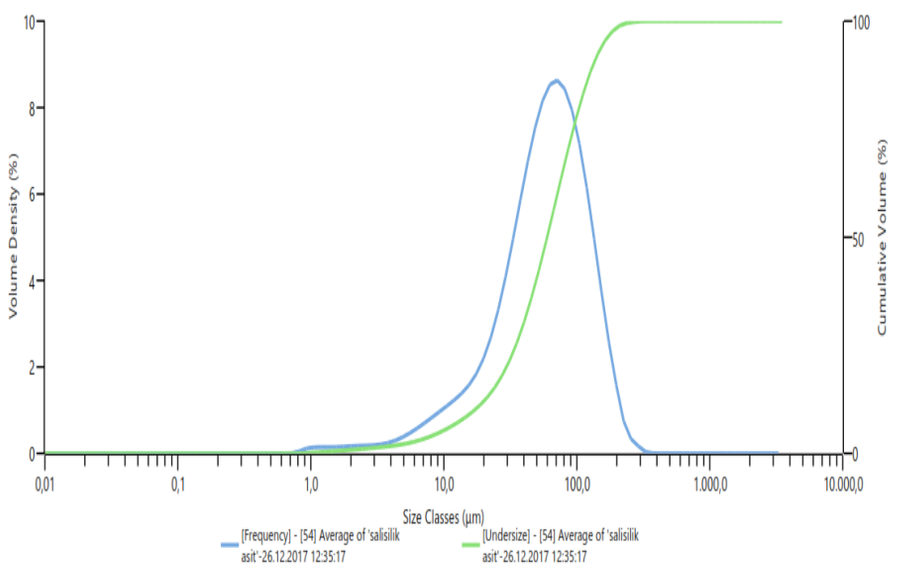


Figure 5. The particle size distribution of **a)** Boric acid and **b)** Salicylic acid.

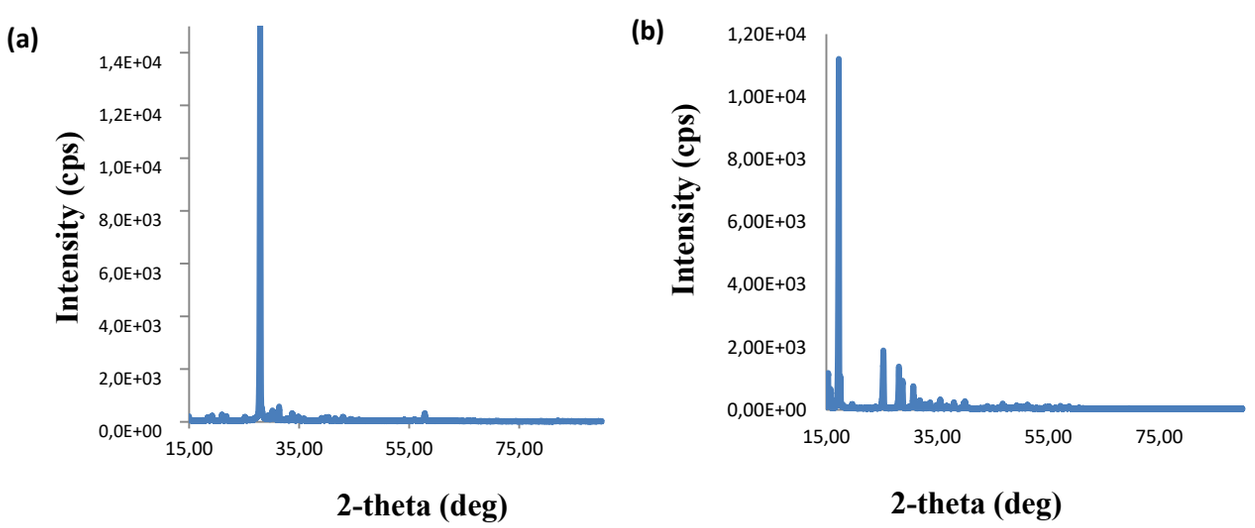


Figure 6. X-ray diffraction pattern for the boric acid **(a)** and salicylic acid **(b)**

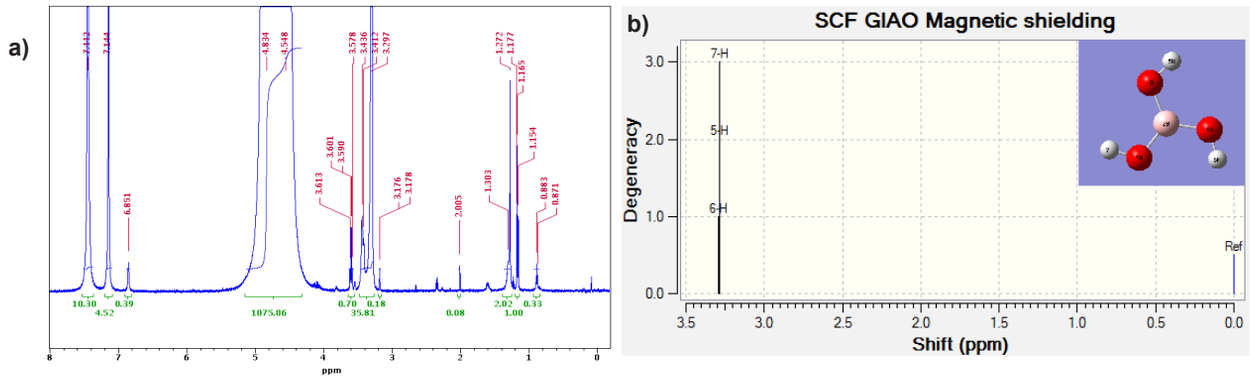


Figure 7. The experimental **a)** and computational **b)** chemical shift spectra of boric acid

The experimental ^{1}H NMR chemical shift spectra of boric acid solution are measured in the range of 1.046-7.838 ppm. The experimental chemical shifts of H atoms, in methanol- d_4, located at 3.79 ppm, 3.73 ppm, 3.72 ppm, 3.71 ppm, 3.62 ppm, 3.60 ppm, 3.59 ppm and 3.58 ppm are in 4H group; the one at 4.99 ppm is in 3H group; those located at 7.84 ppm, 7.46 ppm and 6.93 ppm are in 1H group.

3.5. Antimicrobial activity test

Several studies have been performed on actimicrobial activity determination using a wide variety of bacteria species. Among these species *C. albicans* has a major role on the incidence of invasive fungal infections and poses a serious danger on human health. Sardi et al. [21] reported a review on the epidemiology, biofilm formation, pathogenicity, resistance of Candida species, also related therapies including natural antifungal agents. Hassawi and Kharma (2006) examined the antimicrobial activity of some medicinal plants against C. albicans using the hole-plate diffusion method [22]. Nystatin, used against fungal infections, was applied as control zone. Main choice-drugs for treatment of candidiasis involve antimycotic agents such as polyenes, azoles and antimetabolites agents (i.e. 5-Fluorocytosine, miconazole, amphotericin b, nystatin, fluconazole, and itraconazole) [23-25]. Amphotericin B, flucytosine, ketoconazole, fluconazole, itraconazole, miconazole, griseofulvin, terbinafine, tricomicine and pyramicine are examples to systemic agents; and nystatin, clotrimazole, haloprogin, tolnaftate and naftifine are examples to locally used antifungal agents [26]. In this research, we used nystatin which is known to be effective against *C. albicans,* to investigate the overall effect of our magistral drug.

In this research, agar disk diffusion and agar well diffusion methods were used for determination of the sample's (boric acid solution) antimicrobial activity as given in Figure 9. In the test, a cell suspension in compliance with 0.5 Macfarland turbidity standard and

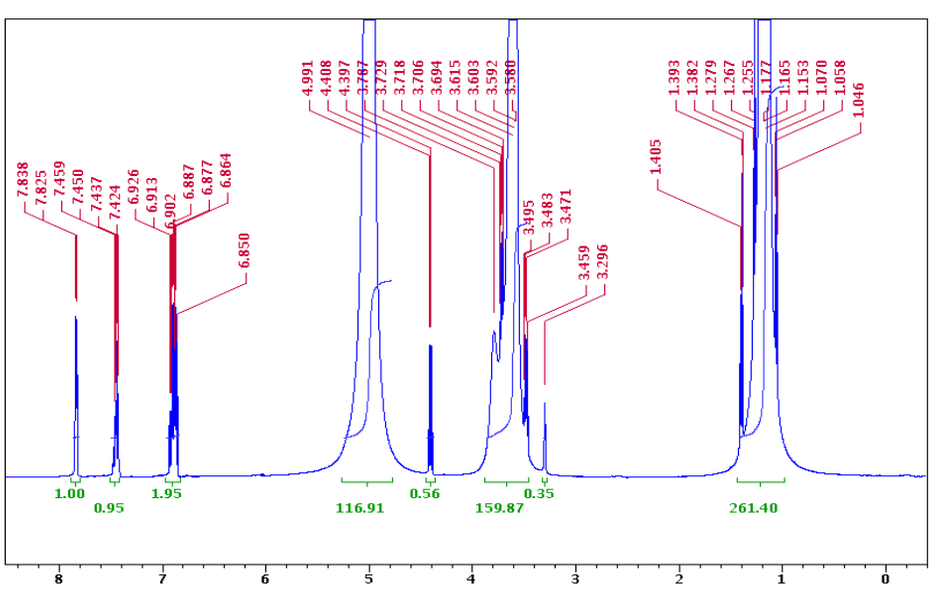


Figure 8. The experimental ^{1}H NMR chemical shift spectra of boric acid solution

Table 2. Antimicrobial activity zone diameter results of the sample (boric acid solution) obtained using *Candida albicans* (*C. albicans* - ATCC 10231) strain

	Disk (20 µl of sample) zone diameter (mm)	Well (20 µl of sample) zone diameter (mm)	Control (Nystatin) zone diameter (mm)
1st Petri	15	11	17
2nd Petri	15	11	17
3rd Petri	14	12	18

prepared from a fresh culture of *C. albicans* –ATCC 10231 strain, was used. In order to ensure turbidity, 4-5 colonies received from a fresh culture of *C. albicans* (24h) was homogenized in a sterile physiological saline solution and the spectrophotometric measurements were performed at OD600. Inoculation from the microorganism suspension (with 0.095 absorbance at OD600) onto Mueller Hinton Agar was made with sterile ecuvion. The disks soaked with 20 µl of the sample were placed onto the agar. 20 µl of the sample was placed into the well which was dented in parallel to the agar. The test was repeated for three times for verification. The petri dishes were incubated for 18-24 hours at 25°C and the diameters of the resulting zones were measured. After the test, antimicrobial activity of boric acid solution was observed on its concentrations tested on *Candida albicans* (ATCC 10231) strain as given in Table 2.

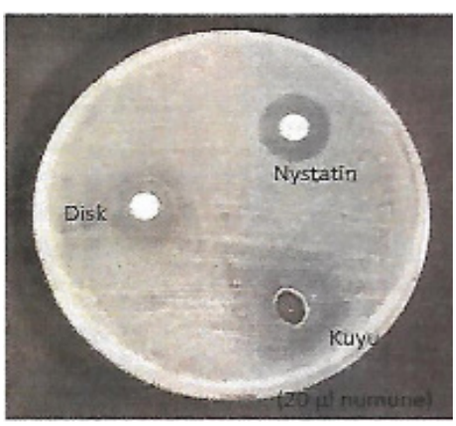


Figure 9. The image of the boric acid solution's zone diameter obtained using *Candida albicans* (*C. albicans* - ATCC 10231) strain

4. Conclusion

In the present study, the optimized structure and vibrational frequencies of boric acid molecule were performed using DFT calculations at the B3LYP/6-311++G(d,p) level. The linear correlation coefficient value (R^2) between the experimental and calculated FT-IR frequencies was found as 0.9941 for IR wavenumbers. Theoretical scaled vibrational frequencies and assignments were found to agree with the experimental FT-IR and NMR shift values in this study. The assignments of the fundamental frequencies were studied on the basis of the calculated and experimental FT-IR and ^{1}H NMR chemical shift spectra. ^{1}H NMR shift analysis of boric acid solution using magistral drug formula was experimentally studied to examine the bonding forms of atoms and molecular structure in the compound. The study supports that boric acid solution has antimicrobial potential against *C. albicans*. Antimicrobial activity zone diameter results of the sample (boric acid solution) on Candida albicans (ATCC 10231) strain were found to be lower than the control zone diameter (Nystatin) in all three measurements, which is indicative of the antimicrobial activity of the tested boric acid solution. Thus, the solution proved to be applicable in the field of healthcare with its antimicrobial property based on the spectroscopic results and antimicrobial activity test.

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