***Balchaturbhadra churna* as a potential medicine for SARS-Cov2 infection in pediatric setting: An In-Silico study**

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Abstract:

Background: Balchaturbhadra Churna (BC), is four ingredients (*Aconitum heterophyllum* Wall. Ex Royle, *Pistacia integerrima* stew. Ex brandis, *Piper Longum* Linn. and *Cyperus rotundus* linn.) Ayurvedic powder dosage form, which is commonly prescribed for treating fever, cough, diarrhea in pediatric setting.

Objective: This present study evaluates the possible effect of phytochemicals from the ingredients of BC on SARS-CoV-2 main protease (M^{Pro}), RNA dependent RNA polymerase (RdRP) and spike-ACE2 (SA) complex through in silico evaluation.

Materials and methods: X-ray crystal structure of M^{Pro} and SA complex with PDB IDs 6LU7 and 6LZG and the SWISS model of RdRP were used as the drug targets. An enhanced docking methodology has been implemented to perform the molecular docking of these phytochemicals against the above-mentioned drug targets of SARS-CoV-2. DOCK 6 was used to perform docking of these phytochemicals. The ADMET and drug likeliness properties for these selected phytochemicals was performed using SWISS ADME.

Results: The ensemble docking studies suggest that aconitine, lappaaconitine from *Aconitum heterophyllum*, 2'-hydroxyisorientin from *Pistacia integerrima* and guineesine, piperidine and piperonic acid from *Piper longum* hold the potential to bind favorably with the SARS-CoV-2 drug targets. The drug likeliness and ADMET properties were calculated for these phytochemicals indicate that all the three phytochemicals from *Piper longum* were observed to have considerate drug-likeliness and ADMET properties. Aconitine has molecular weight slightly higher than 500 kDa and has more than 10 hydrogen bond acceptors.

Conclusion: The in-silico study demonstrates that phytochemicals from the ingredients BC viz., aconitine, lappaaconitine from *Aconitum heterophyllum*, 2'-hydroxyisorientin from *Pistacia integerrima* and guineesine, piperidine and piperonic acid from *Piper longum* hold the potential to bind favourably with the SARS-CoV-2 drug targets. The study also provides evidence for drug likeliness and ADMET properties for these phytochemicals suggesting that BC can be further studied as potential drug of choice for treating SARS-CoV-2 infection in paediatric cases.

Keywords: COVID-19, SARS-CoV-2, Balchaturbhadra, Molecular Docking, *Aconitum heterophyllum*, *Pistacia Integerrima*, *Piper Longum*, *Cyperus rotundus*, Ativisha, Shringi, Pippali, Musta, Ayurveda, in-silico study, ensemble docking

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Introduction

Coronavirus disease 2019 (COVID-19) is caused by highly contagious virus strain SARS-Coronavirus 2 (SARS-CoV-2), mainly characterized by fever and respiratory symptoms. Complications such as acute respiratory distress syndrome (ARDS) and death are primarily seen in the elderly, and in Covid-19 individuals with co-morbidities for example hypertension, obesity or chronic kidney disease¹. In comparison with adults, the global incidence of Covid-19 disease in children is strikingly lower. Children of all ages can get COVID-19 but those younger than 12 to 14 years of age appears to be affected less commonly than adults. In surveillance from various countries children account for up to 15% of laboratory confirmed SARS-CoV-2 cases².

The lower observed prevalence of Covid-19 in pediatric age group worldwide is partially attributed to widespread school closures in response to the pandemic. Furthermore, challenges in the adequate screening and testing of children especially those who are asymptomatic and mildly symptomatic may have underestimated the reporting of covid-19 cases in children^{3,4}. The symptoms of COVID-19 are similar in children and adults, but the frequency of symptoms varies. In general, 90% of children positive for SARS-CoV-2 infection are asymptomatic or have mild to moderate symptoms with better outcome^{5,6}. In rare cases, a post infectious disease, characterized by multisystem inflammatory syndrome in Children (MIS-C), Kawasaki – like symptoms and acute heart failure can occur, but in general children have a very low risk of death⁷.

India has seen a devastating second wave due to the Delta variant with increased morbidity and mortality compared with first wave⁸. There were media reports of drastic increase in number of cases in children with SARS-CoV-2 infection in comparison to first wave, and with more concerns of rising post infectious life-threatening complications in children 2 – 4 weeks after contracting Covid-19 infection^{9–11}.

As the second wave of Covid-19 was ebbing amid accelerated vaccination drive among adult and elderly population, India has recently seen a sharp rise in Covid-19 cases over the past few weeks. At the time of writing current manuscript, daily cases increased from 6,531 on Dec. 26, 2021, to 2,38,018 on Jan. 17, 2022, a 36-fold increase in 22 days, which has sparked a fear that a third wave driven by Omicron is around the corner¹².

The World Health Organization (WHO) on November 26, 2021 declared the Omicron (B.1.1.529 strain of Covid-19), first detected in South Africa, to be a variant of concern due to several mutations including 15 mutations in the receptor-binding domain (RBD) of spike protein as well as increased transmissibility. Several studies have raised concern about Omicron evading the antibodies thus increasing the chances of reinfection as well as breakthrough infections. Nevertheless, the findings are of preliminary nature and more clarity will be evident in coming weeks^{13,14}.

Govt. of India has rolled out Covid-19 vaccination drive for children in staggered manner phase with first group of 15 – 18 years of age getting jab from 3rd January, 2022. Amid sharp rise in cases, there are reports of rising infection in young children with many of those infected by the virus showing symptoms such as fever, cough, chills, diarrhea and

respiratory problems. Experts believe that the actual number of positive cases may be under reported due to less number of RT - PCR test in young children^{15,16}.

The COVID-19 pandemic has considerably increased the demand to develop faster therapeutics to overcome any such global emergency. Ayurveda, being one of the most trusted modes of traditional medicine has proved to be a boon for patients recovering from COVID-19.

To note, AYUSH system has considerably played a major role by undertaking several R&D activities and public health initiatives to harness the potential of AYUSH systems to contain the impact of the COVID-19 pandemic¹⁷. To its recent success, various interventional clinical trials undertaken in asymptomatic, mild to moderate COVID-19 infections have shown positive results in combating covid-19 infection. In this context, multicentric clinical trial of Ayurvedic formulation Ayush -64 (developed by Central Council for Research in Ayurvedic Sciences, New Delhi) as add on to Standard care of treatment have shown early clinical recovery response in Covid-19 contracted patients¹⁸.

With the lessons learned from second wave, the Government of India has taken several steps at various levels to ramp up the existing health care facilities for children to prepare for sudden surge of COVID-19 cases in the pediatric age group. In this context, the Ministry of Health and Family Welfare (MOHFW) and Ministry of Ayush (MoA), two pillars of Indian health care system to Govt. of India have issued operational guidelines and advisories for prevention and mitigation of Covid-19 in children^{19,20}. MoA released home care guidelines for children and advisory for AYUSH practitioners about prophylactic care in children during the covid-19 pandemic. Formulations like Ayush-64, Balchaturbhadra churna, Sitopaladi churna etc. have been incorporated for symptomatic care of covid-19 pediatric patients²¹.

Balchaturbhadra churna (BC) is a polyherbal formulation mentioned in classical Ayurvedic texts specifically indicated for fever (Jvara), cough (Kasa) and diarrhea (Atisaara) in children. It consists of four ingredients viz., Aruna=Ativisha (*Aconitum heterophyllum* Wall. Ex Royle), Shringi (*Pistacia integerrima* stew. Ex brandis), Krisna=Pippali (*Piper Longum* Linn.) and Ghana=Musta (*Cyperus rotundus* linn.)²²⁻²⁵.

The Ayurvedic pharmacological profile of individual medical plants used in BC is provided in table number two²⁶⁻³⁰. To summarize it, the formulation is characterized predominantly by katu (pungent) – tikta (bitter) rasa (taste), kapha-vata dosha har (pacifies kapha and Vata dosha), ushna veerya (hot potency), and with deepana (digestion and metabolism enhancer) – pachana (digestive enhancer) properties it is therefore indicated in Jvara (fever), Kasa (cough) and Atisaara (diarrhea). The combined effects of these herbs render this compound as a potent combination against conditions presenting with symptoms of cough/cold, fever and diarrhea, as found in Flu-like Viral Illnesses.

The chemical compounds found in the ingredients of BC have been reported to exhibit several pharmacological properties like anti-pyretic, anti-inflammatory, anti-oxidant, anti-diarrheal, anti-platelet etc. (see table no.3)³¹⁻⁶⁵. Pre-clinical pharmacological and toxicological studies have been undertaken to study its safety aspect (table no.4). The acute toxicity experiment did not produce any signs and symptoms of toxicity up to the dose of 2000mg/kg but at dose of 900mg/kg given for 45 days has shown mild toxic potential⁶⁶.

In order to understand the role of these formulations in targeting the crucial proteins of SARS-CoV-2 an in-silico molecular docking study has been performed. The phytochemicals that are present in the formulation of BC have been docked against three important drug targets of SARS-CoV-2, namely, main protease (M^{Pro}), RNA dependent RNA polymerase (RdRP) and spike protein. The spike protein of SARS-CoV-2 binds to the ACE2 receptor present on the human cells. Hence, the been considered as the target in the present study. The X-ray crystal structure of M^{Pro} and SA complex with PDB IDs 6LU7 and 6LZG and the SWISS model of RdRP were used as the drug targets⁶⁷⁻⁶⁹. Figure no. 1 shows the structure of these three drug targets along with the drug-binding region marked in red dashed circle.

Figure 1:(A) M^{Pro} bound to N3 as in 6LU7, (B) RdRP with RNA primer binding region circled in red and (C) SA complex from 6LZG with the interaction site circled in red.

These three drug targets were docked using a set of 32 phytochemicals that belonged to the formulation of BC. These phytochemicals are isolated from four of BC ingredients, namely, *Aconitum heterophyllum*, *Pistacia integerrima*, *Piper longum* and *Cyperus rotundus*. The 2D representation for these phytochemicals as given in PubChem database has been shown in the Figure 2.

Figure 2: Name and PubChem Compound ID (CID) (in brackets) of the phytochemicals present in the BC (*Balchaturbhadra churna*)

An enhanced docking methodology has been implemented to perform the molecular docking of these phytochemicals against the above-mentioned drug targets of SARS-CoV-2. This methodology has been termed as ensemble docking, where in multiple structural instances of the drug targets are docked instead of just a single experimental or computational model. This methodology has been reported in few of our earlier works^{70–72}.

Methodology

The methodology used to perform ensemble docking has been adapted from three of our earlier works on in-silico docking of small molecules to M^{Pro}⁷⁰, RdRP⁷¹ and SA complex⁷². Figure 3 explains this methodology in details.

Figure 3: Protocol followed for identifying the best docked phytochemicals of BC (*Balchaturbhadra churna*)

The simulation data generated from the step mentioning “MD simulation of the protein in the apo-state” was used from three of our previous works^{70–72}. AMBER 16 simulation package was used for these simulations⁷³. A total of 16, 5 and 5 ensemble representatives of M^{Pro}, RdRP and SA complex were docked using the 32 phytochemicals of *Balchaturbhadra churna*. DOCK 6 was used to perform docking of these phytochemicals⁷⁴. The rigid docking protocol was followed for the same. DOCK 6 assigns a grid score for each of the molecules that are docked against the drug targets. These grid scores are energy values and hence, lower the energy better is the binding of the molecules to the drug targets. The grid score is calculated using the force field equation as given below.

$$E = \sum_{i=1}^{\text{lig}} \sum_{j=1}^{\text{rec}} \left(\frac{A_{ij}}{r_{ij}^a} - \frac{B_{ij}}{r_{ij}^b} + 332 \frac{q_i q_j}{D r_{ij}} \right)$$

E is the grid score, i and j are the number of atoms belonging to ligands (phytochemicals), denoted as lig and receptor (drug targets) denoted as rec, respectively. $\frac{A_{ij}}{r_{ij}^a} - \frac{B_{ij}}{r_{ij}^b}$ represents the contribution due to van der Waals interactions. The term $332 \frac{q_i q_j}{Dr_{ij}}$ represents the contribution due to electrostatics interactions.

DOCK 6 ranks these phytochemicals based on their grid scores in each of the ensembles. Apart from ensemble docking, direct docking to the experimental and computational models known through literature (Figure 1) was also performed. Based on these grid scores, the phytochemicals that ranked 1st in either of the ensembles or the experimental/computational model were selected. There were instances where some phytochemicals were either ranked 1st in more than one ensemble or not ranked 1st in any ensemble. The interaction analysis for such instances were performed using the LigPlot software⁷⁵. The ADMET and drug likeliness properties for these selected phytochemicals was performed using SWISS ADME⁷⁶.

Results

Targeting the MPro

The selected phytochemicals that showed the best grid score in either of the ensembles or the experimental/computational model were identified. Figure 4 shows the grid scores for the selected phytochemicals when docked against M^{Pro} and the residues that show interactions with these selected phytochemicals.

Figure 4: (A) Ligands with best grid score obtained on docking to either of the 16 ensembles or the 6LU7 (marked in red) of MPro. Interactions between (B) aconitine, (C) 2'-hydroxyisoorientin, (D) guineesine, (E) piperidine and (F) pipericoic acid

The ensemble docking in case of MPro consisted of 16 ensemble states captured through Root Mean Square Deviation (RMSD)-based clustering and Markov State Modeling (MSM)-based clustering⁶⁷. One of our earlier research articles, mentions the details about these 16 ensembles (Figure 5 of Koulgi et.al. 2020)⁶⁷. The 32 phytochemicals from BC were docked individually on these 16 ensembles and also on the X-ray crystal

structure 6LU7. The phytochemicals aconitine, 2'-hydroxyisoorientin, guineesine, piperidine and pipericoic acid ranked 1st in either one of the ensembles or 6LU7. Figure 4A shows the best grid scores obtained. Aconitine, 2'-hydroxyisoorientin, and guineesine had the best grid scores amongst the 16 ensembles. However, piperidine and pipericoic acid had the best grid scores when docked to 6LU7. Figure 4B-F show the interactions made by aconitine, 2'-hydroxyisoorientin, guineesine, piperidine and pipericoic acid with the residues of M^{Pro}. The red color represents hydrophobic interactions and the hydrogen bonding interactions have been represented by green dashed lines. Aconitine was observed to form a hydrogen bond with T190 of M^{Pro} (Figure 4 B). 2'-hydroxyisoorientin formed a hydrogen bond with Q189 and Q192 of M^{Pro} (Figure 4 C). Guineesine and piperidine formed only hydrophobic interactions with residues of M^{Pro} (Figure 4D and E). R188 formed a hydrogen bond with pipericoic acid (Figure 4F). These interactions were seen in docked states with most favorable grid scores. Hence, it can be inferred that these interactions are energetically favorable and may prove beneficial for bringing in the inhibitory effect.

Targeting the RdRP

Figure 5 shows the grid scores for the three phytochemicals selected when docked against RdRP and their interaction with the residues of this drug target.

Figure 5: (A) Ligands with best grid score obtained on docking to either of the 5 ensembles of RdRP. Interactions between (B) lappaconitine, (C) aconitine, and (D) guineesine.

In case of ensemble docking to RdRP protein of SARS-CoV-2, a total of 5 ensemble representatives were considered. One of our earlier works on drug repurposing studies with RdRP mentions about the method used to generate these ensemble representatives⁶⁸. Among the 32 phytochemicals from the BC, only 3 of them ranked 1st in either of the 5 ensemble representatives. The value of the grid score was also observed to be lower in them as compared to the ones obtained on docking to the computational model. These three phytochemicals were lappaconitine, aconitine and guineesine. Lappaconitine and aconitine were observed to form multiple hydrophobic interactions with the residues of the RdRP (Figure 5 B and C). Guineesine was observed to form hydrogen bonding with R555 of

Drug likeliness of phytochemicals

The ensemble docking studies suggest that aconitine, lappaaconitine from *Aconitum heterophyllum*, 2'-hydroxyisorientin from *Pistacia intergerrima* and guineesine, piperidine and piperolic acid from *Piper longum* hold the potential to bind favorably with the SARS-CoV-2 drug targets. The medicinal properties of these plants have been known since long in traditional medicine, hence, understanding the drug likeliness of the phytochemical would prove beneficial to support their potential as SARS-CoV-2 inhibitors⁷⁸⁻⁸². The drug likeliness and ADMET properties were calculated for these phytochemicals. Table 1 shows these details for the above-mentioned phytochemicals. Aconitine has molecular weight slightly higher than 500 kDa and has more than 10 hydrogen bond acceptors. Expect these most of the other parameters are observed to be considerable. All the three phytochemicals from *Piper longum* were observed to have considerable drug-likeliness and ADMET properties.

Conclusion:

Considering the possibility of pediatric population getting affected in the possible third wave of COVID-19 pandemic there is a need to identify potential, safe medicines. BC, an Ayurvedic powder dosage form is a regularly prescribed medicine to treat fever, cough, diarrhea in pediatric setting. Further, it has been included in the treatment protocols released by Ministry of Ayush for treating asymptomatic, mild cases of COVID-19. The in-silico study demonstrates that phytochemicals from the ingredients BC viz., aconitine, lappaaconitine from *Aconitum heterophyllum*, 2'-hydroxyisorientin from *Pistacia intergerrima* and guineesine, piperidine and piperolic acid from *Piper longum* hold the potential to bind favorably with the SARS-CoV-2 drug targets. The study also provides evidence for drug likeliness and ADMET properties for these phytochemicals suggesting that BC can be further studied as potential drug of choice for treating SARS-CoV-2 infection in pediatric cases.

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Conflicts of Interest: The authors declare no conflict of interest

Table no. 1: Ingredients of Balchaturbhadra churna²² -

Sl. No.	Name of Ingredients	Latin name	Family	Parts used	Proportion
1	Aruna (Ativisha)	<i>Aconitum Heterophyllum</i> Wall. Ex Royle	Ranunculaceae	Root	1 part
2	Shringi (Karkatashringi)	<i>Pistacia Integerrima</i> stew. Ex Brandis	Anacardiaceae	Gall	1 part
3	Krisna (Pippali)	<i>Piper Longum</i> Linn.	Piperaceae	Fruit	1 part
4	Ghana (Musta/Nagarmotha)	<i>Cyperus rotundus</i> Linn.	Cyperaceae	Rhizome	1 part

(Reference - As per The Ayurvedic Formulary of India (AFI, Part- I)7: 24 Balchaturbhadrika churna (Bhaisajyaratnavali, Balarogadhikara:40)

Table no. 2: Ayurvedic pharmacological profile of medicinal plants in Balchaturbhadra churna²⁶⁻³⁰:

Name of medicinal plant	Rasa (taste)	Guna (properties)	Virya (potency)	Vipaka (Bio-transformed rasa)	Doshagh nata (pacifies Dosha)	Karma (Therapeutic action)	Vyadhi har (Curative)
<i>Aconitum Heterophyllum</i> Wall.	Katu (pungent) Tikta (bitter)	Laghu (lightness), Ruksha (dryness)	Ushna (hot)	Katu (pungent)	Kapha – Pitta har (pacifies Kapha & Pitta dosha)	Pachana (digestion and metabolism enhancer), Deepana (digestive enhancer)	Atisara (diarrhea), Ama, Vish (poison/toxins), Kasa (cough), Krimi roga (worm infestation), Chardi (vomiting), Amatisara (diarrhea associated with Ama)
<i>Pistacia Integerrima</i> stew. ex Brandis	Tikta (bitter) Kashaya (astringent)	Guru (heaviness)	Ushna (cold)	Katu (pungent)	Kapha-Vata har (pacifies Kapha & Vata dosha)	Kasahara (cough reliving) Hikkanigra hana (hiccough relieving)	Jwara (pyrexia), Shwasa (breathing difficulty), Urdhava

							(obstruction of Vata in the intestine/ reverse peristalsis), Trita (thirst/polidipsia), Kasa (cough), Hikka (hiccup), Aruchi (anorexia), Chardhi (vomiting), Atisara (diarrhoea), Raktapitta (bleeding disorders)
<i>Piper Longum</i> Linn.	Katu (pungent), Tikta (bitter)	Laghu (lightness), Snigdha (unctuousness), ushna (hotness)	Anushna (neither hot nor cold)	Madhura (sweet)	Vata-Kapha har (pacifies Vata&Kaphadosha)	Deepana (digestive enhancer), Vrisya (aphrodisiac), Rasayana (rejuvenator), Rechaka (osmotic laxative), Pittala (increases Pitta)	Swasa (difficulty in breathing), Kasa (cough), Udarroga (ascites), Jwara (pyrexia), Kustha (integumentary diseases), Prameha (diabetes mellitus), Gulma (lump), Arsha (haemorrhoids), Pliharoga (splenic disorders), Shula (pain), AamMaruta (rheumatism due to Ama), Kshayaroga (emaciation)
<i>Cyperus rotundus</i> Linn.	Katu (pungent),	Laghu (lightness), Ruksha (dryness)	Sheeta (cold)	Katu (pungent)	Kapha-Pitta-Rakta-har	Grahi (absorptive), Pachana	Trishna (thirst/polidipsia), Jwara (pyrexia),

	Tikta (bitter), Kashay (pungent)				(pacifies Kapha, Pitta and Raktados ha)	(digestion and metabolism enhancer), Deepana (digestive enhancer), Swedjanak a (perspiratio n enhancer), Mutra- janaka (diuretic), Stanya- vardhaka (galactogo gue), artava- janak (emmenag ogue), Kesh- vardhak (promotes hair growth), Vrana- ropaka (wound healer)	Aruchi (anorexia), Krimihar (worm infestation), Atisara (diarrhoea),
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Table no.3: Pharmacological activity of Balchaturbhadrachurna by its ingredients³¹⁻⁶⁵

Medicinal Plant	Pharmacological activities
<i>Aconitum Heterophyllum</i> Wall. ^{31,32,43,54}	anti-inflammatory, anti-bacterial, anti-oxidant, anti-diarrhoeal
<i>Pistacia Integerrima</i> stew. Ex Brandis ⁶⁰⁻⁶⁴	anti-pyretic, anti-spasmodic, anti-diarrheal, anti-asthmatic, anti-inflammatory, analgesic,
<i>Piper Longum</i> Linn. ^{33-42,44-46,65}	anti-viral, anti-inflammatory, anti-tumor, neuroprotective, anti-asthmatic, anti-oxidant, hepatoprotective, immunomodulator, anti-platelet, analgesic, bioavailability enhancer, cardioprotective, neuroprotective, antibacterial
<i>Cyperus rotundus</i> Linn. ^{47-53,55-59}	anti-oxidant, anti-ulcer, analgesic, anti-microbial, cytotoxic, anti-convulsant, anti-diabetic, anti-diarrheal, anti-spasmodic, anti-inflammatory, hepatoprotective,

	cardioprotective, anti-lipidemic, anti-platelet, anti-depressant, neuroprotective
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Table no. 4: Preclinical Pharmacological and toxicological/ safety studies of Balchaturbhadra churna:⁶⁶

Sl. No.	Pre-clinical pharmacological and toxicological / safety studies
1.	In albino-wistar rats, the acute toxicity experiment showed that the drug did not produce any signs and symptoms of toxicity (or mortality) up to the dose of 2000 mg/kg
2.	<i>Balacaturbhadra churna</i> at dose of 900 mg/kg given for 45 days has shown mild toxic potential.

Table no. 5: ADMET properties of the selected phytochemicals from *Aconitum heterophyllum*^{*1}, *Piper longum*^{*2} and *Pistacia intergerrima*^{*3}

Molecule	Molecular weight (kDa)	No. of H-bond acceptors	No. of H-bond donors	Total Surface Area	Lipophilicity (WLOGP)	Solubility	GI absorption	BBB permeability	P-gp substrate	CYP3A4 Inhibitor	log K _p (cm/s)	Lipinski# violations
Acotinine ^{*1}	645.74	12	3	153.45	0.27	Soluble	Low	No	Yes	No	-10.04	2
2'-hydroxysoorientin ^{*3}	465.38	12	10	228.6	-1.12	Soluble	Low	No	No	No	-9.5	2
Guineesine ^{*2}	383.52	3	1	47.56	5.54	Moderately soluble	High	Yes	No	Yes	-3.8	0
Pipercide ^{*2}	341.44	3	1	47.56	4.52	Moderately soluble	High	Yes	No	Yes	-4.05	0
Piperoic acid ^{*2}	358.47	4	3	77.76	5.76	Very soluble	High	Yes	No	No	-6.52	0
Lappaconitine ^{*1}	584.7	9	3	126.79	1.65	Soluble	High	No	Yes	No	-8.74	1

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