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Evaluation of *in silico* anthelmintic activity of Naaku Poochi Kolli Kudineer Chooranam, a Siddha formulation, against glutamate-gated chloride ion channel, β-tubulin, and nicotinic acetylcholine receptor, and ADMET profile

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ABSTRACT

Background: Helminthiasis in humans and livestock causes nutritional deficiency, immunosuppression, and poor productivity. The emergence of resistance to conventional anthelmintic drugs such as ivermectin, albendazole, and oxantel necessitates alternative therapeutic strategies. Naaku Poochi Kolli Kudineer Chooranam (NPK), a traditional Siddha polyherbal formulation, is reputed for its worm-expelling properties, but its mechanism of action remains unexplored. **Objective:** This study aimed to investigate the *in silico* anthelmintic potential of NPK phytochemicals against key helminthic targets-glutamate-gated chloride ion channel (GluCl), β-tubulin, and nicotinic acetylcholine receptor (nAChR), and to assess their pharmacokinetic and toxicity profiles. **Materials and Methods:** Phytochemicals of NPK were retrieved from the IMPPAT database and screened for physicochemical suitability (PAINS = 0, logP 1.5–4.5). Homology models of Ascaris suum β-tubulin and nAChR ACR-16 were generated using MODELER, while the GluCl structure was obtained from PDB (3RIF). Molecular docking was performed using Discovery Studio 2020 with ivermectin, albendazole, and oxantel as reference drugs. ADMET and toxicity profiles were predicted using Discovery Studio modules. **Results:** Of the 1687 phytochemicals screened, 225 compounds met the selection criteria. Several, including ellagic acid, luteolin, silibinin, and butein showed higher binding affinities than the reference drugs. Ellagic acid exhibited multi-target activity, binding strongly to GluCl (-101.7 kcal/mol), β-tubulin (-95 kcal/mol), and nAChR (-117.1 kcal/mol). The top compounds interacted with key residues associated with anthelmintic activity. ADMET analysis revealed that butein, butin, 2-methyl-3-glucosyloxy-5-isopropyl phenol, acetyleugenol, acetovanillone, luteolin, nigellicine, and silibinin possessed good solubility, absorption, and non-carcinogenic profiles without predicted hepatotoxicity. **Conclusion:** The study provides molecular evidence supporting the traditional anthelmintic use of NPK. Its phytochemicals effectively target GluCl, β-tubulin, and nAChR, suggesting potential mechanisms involving neuromuscular paralysis of parasites. These findings identify several bioactive compounds as promising, safe candidates for further *in vitro* and *in vivo* validation as next-generation plant-derived anthelmintics.

Keywords: Naaku Poochi Kolli Kudineer Chooranam, Siddha medicine, Molecular docking, ADMET, Anthelmintic, GluCl, β-tubulin, nAChR.

INTRODUCTION

Helminthiasis affects 2 billion people worldwide, causing anemia and nutritional and cognitive deterioration due to poor absorption and availability of nutrients in the host [1]. Major intestinal parasitic diseases in humans and ruminants, such as ascariasis, trichinellosis, and strongyloidiasis, are caused by nematodes, sparganosis, cysticercosis, echinococcosis by cestodes, fascioliasis, clonorchiasis, and schistosomiasis by trematodes [2]. Various classes of anthelmintics such as macrocyclic lactones, benzimidazoles, imidazothiazoles, and tetrahydropyrimidines are commonly used for prophylaxis. However, the use of these classical drugs is threatened by the development of drug resistance, which has evolved since the early 1980s in various parasitic species [3,4]. Alternate strategies need to be developed to combat and reduce the morbidities caused by helminth diseases and anthelmintic resistance.

The use of medicinal plants in traditional medicine has been prevalent since time immemorial and they serve as natural sources of compounds with therapeutic potential. The traditional Indian anthelmintic Siddha formulation, Naaku Poochi Kolli (NPK) kudineer chooranam, is used to treat parasitic diseases in humans and comprises of *Butea monosperma, Nigella sativa, Embelia ribes, Operculina turpethum, Cassia angustifolia, Trachyspermum ammi, Picrorhiza kurroa, Pimpinella anisum, and Foeniculum vulgar*. NPK has equal efficacy to ivermectin and albendazole against the intestinal parasite Syphacia in rats [5], and *in vitro* efficacy against *Haemonchus contortus,* prompting uterine contraction and expulsion

of eggs from female worms [6]. Except for *O. turpethum*, the anthelmintic activity of all individual plants in the formulation has been reported in *in vitro* and *in vivo* studies [7,8,9,10,11,12]. However, to the best of our knowledge, the mechanism of action of this formulation has not been investigated.

The drug targets of commonly used anthelmintics, such as ivermectin, oxantel, and albendazole, are well established to be glutamate-gated chloride ion channels (GluCl), nicotinic acetylcholine receptors (nAChR), and beta-tubulin, respectively [13, 14, 15, 16], the *in silico* interaction of these targets with phytochemicals would provide insights into the mechanism of action of the herbal formulation. Using ivermectin, albendazole, and oxantel as positive controls, this study aimed to investigate the binding modes and interactions of the phytochemicals of the NPK formulation with the targets GluCl, beta-tubulin, and N-type nAChR, as well as their pharmacokinetic profiles, to identify potential drug candidates from the formulation.

MATERIALS AND METHODS

Identification of targets and molecular modeling

The glutamate-gated chloride channel structure (PDB:3RIF) was obtained from the PDB. Homology modeling of *Ascaris suum* β-tubulin (UniProt:F1L8A5) and nAChR ACR-16 (UniProt:F1KYJ9) was performed using MODELER (BIOVIA, 2020). Templates were selected via NCBI BLASTp, and 100 models were generated and evaluated using verification scores, DOPE scores [17, 18], and Ramachandran plots.

Selection of ligands

The phytochemicals of the NPK formulation were sourced from IMPPAT [19]. The compounds were filtered for PAINS=0 and logP (1.5–4.5) [20, 21], ≥1 aromatic ring, and ≥1 H-bond donors/acceptors. The structures of the clinical drugs, albendazole, oxantel, and ivermectin, were retrieved from PubChem and ChEMBL. Ligands were prepared using Discovery Studio 2020's '*Prepare Ligand'* protocol to rectify valencies/duplicates.

Molecular docking

Molecular docking was carried out with CDOCKER in Discovery Studio 2020 [22], validated by redocking ivermectin to GluCl (PDB:3RIF; RMSD:1.4–1.8Å). Ivermectin (GluCl), albendazole (β-tubulin), and oxantel (nAChR) were docked as positive controls. The binding energies, H-bonds, hydrophobic contacts, and unfavorable interactions were analyzed for all complexes.

In silico ADMET Predictions

DS 2020 Toxicity Prediction (carcinogenicity/ mutagenicity/ teratogenicity) and ADME descriptor (aqueous solubility, hepatotoxicity, CYP450 inhibition, BBB permeability, and plasma protein binding) modules were used to assess the drug-like potential of the compounds.

RESULTS

Homology modelling

The *Ascaris suum* β-tubulin-2 (F1L8A5) was modeled using *Bos taurus* (PDB:5IYZ) (95% identity, 98.8% similarity) with a 0.2 Å RMSD and 99% favored/allowed Ramachandran residues. The nAChR ACR-16 (F1KYJ9) dimer was modeled using human α7 receptor (PDB:7EKI) (55.5% identity, 75.4% similarity), and had a 0.7 Å RMSD and 97% favored/allowed residues. Both models were selected based on their optimal verification and negative DOPE scores, confirming their structural reliability.

Active site prediction

The active sites of the prepared proteins were identified using DS 2020. For GluCl, the ivermectin-binding site in the transmembrane domain was defined on the basis of the binding site determined by Hibbs (2011) [26] (Figure 1a). In nAChR, the ligand-binding domain present in the extracellular domain was defined at the orthosteric binding site [23] (Figure 1b, Figure 2a, 2b), and the binding site in beta-tubulin was selected from the receptor cavities (Figure 1c).

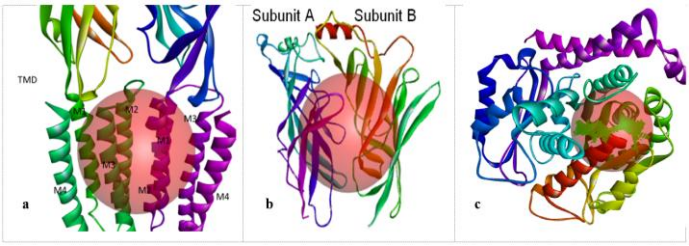


Figure 1: Binding sites of GluCl, nAChR and beta-tubulin. **(a)** Transmembrane domains (TMD) M1, M2, M3, and M4 of the two adjacent subunits of glutamate-gated chloride ion channel with ivermectin binding site spheres. **(b)** Orthosteric binding site of nAChR between the extracellular domains of two adjacent subunits within the site sphere. **(c)** Binding site of beta-tubulin within site sphere.

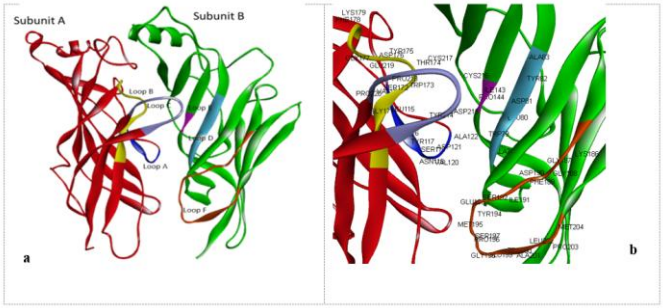


Figure 2: Orthosteric binding site of nAChR. **(a)** nAChR- loops comprising the orthosteric binding site. **(b)** nAChR- loop residues present in the orthosteric binding site.

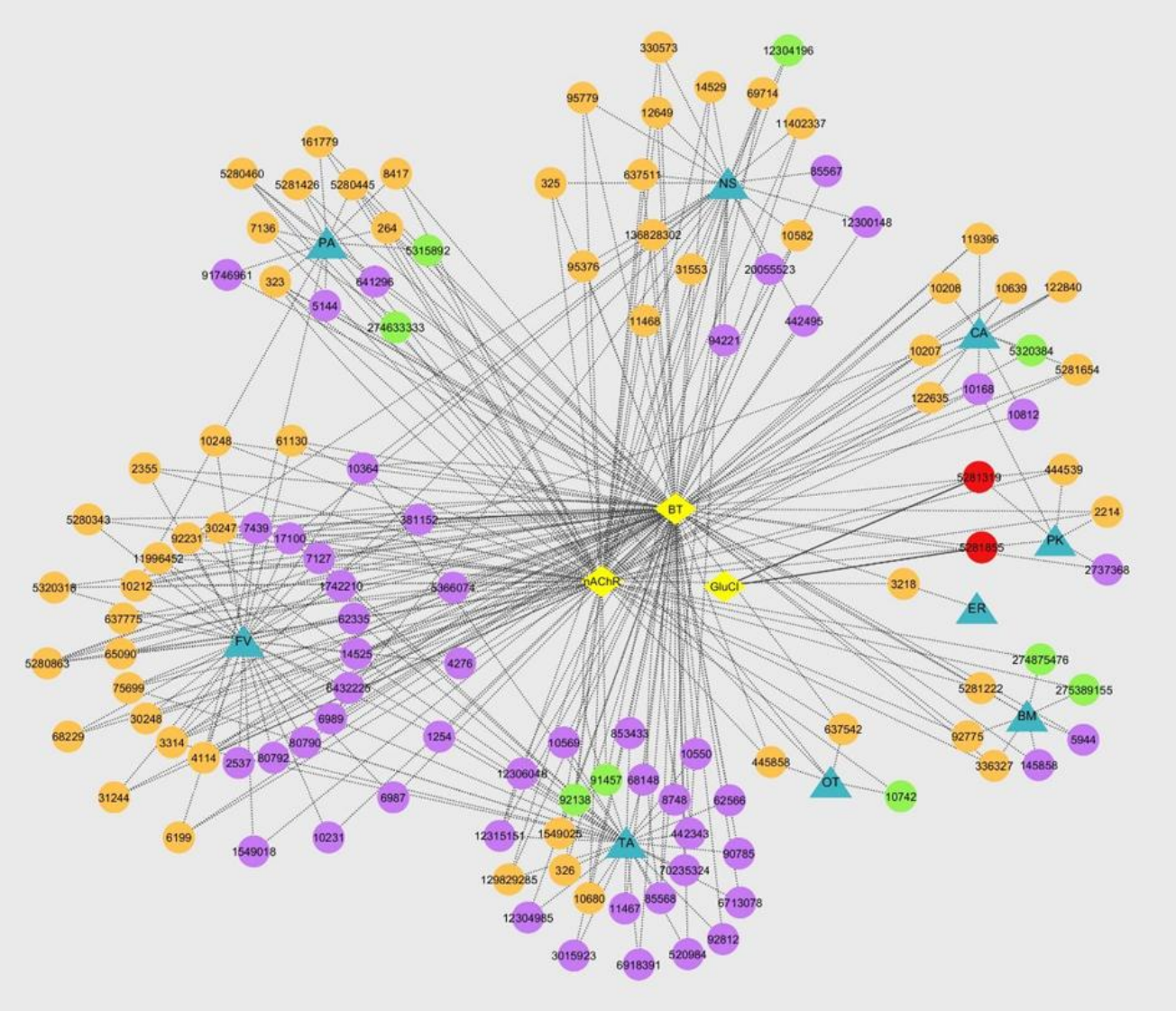
Selection of ligands

A total of 1687 phytochemicals were obtained from the IMPPAT database for NPK herbs. The compounds were reduced to 225 after screening for the presence of an aromatic ring, PAINS score (zero alerts), logP (1.5-4.5) and the presence of hydrogen acceptors and donors.

Molecular docking and binding energy

Molecular docking with CDOCKER protocol revealed 133, 188, and 187 NPK compounds bound GluCl, β-tubulin, and nAChR respectively, with 2, 134, and 57 compounds showing higher binding energies that ivermectin (-82.8 kcal/mol), albendazole (-38.7 kcal/mol), and oxantel (-47.7 kcal/mol). Ellagic acid exhibited a high affinity for all three targets. A compound-target interaction network was created using Cytoscape 3.0 (Figure 3), and the interactions of the top ten highest-scoring compounds for each protein target were analyzed (Table 1).

Figure 3 compound-target interaction network of compounds with binding energies higher than those of the drug controls. PubChem IDs of the compounds interacting with the targets are represented by dotted lines and purple circles for beta tubulin, dotted lines and green circles for nAChR, dotted lines and orange circles for both nAChR and beta tubulin interactions, and solid lines and red circles for GluCl, nAChR, and beta tubulin. No interactions were observed when GluCl was used alone. Blue triangles represent plants, and yellow diamonds represent the three targets beta tubulin, GluCl, and nAChR.



BT- Beta tubulin, BM- *Butea monosperma*, ER-*Embelia ribes*, OT-*Operculum turpethum*, FV-*Foeniculum vulgare,* PK- *Picrorhiza kurroa*, PA-*Pimpinella anisum*, CA- *Cassia angustifolia*, TA- *Trachyspermum ammi*

Figure 3: Compound-target interaction network of compounds with binding energies higher than those of the drug controls. PubChem IDs of the compounds interacting with the targets are represented by dotted lines and purple circles for beta tubulin, dotted lines and green circles for nAChR, dotted lines and orange circles for both nAChR and beta tubulin interactions, and solid lines and red circles for GluCl, nAChR, and beta tubulin. No interactions were observed when GluCl was used alone. Blue triangles represent plants, and yellow diamonds represent the three targets beta tubulin, GluCl, and nAChR.

Pharmacokinetics

The ADMET profiles of the compounds displaying higher binding energies than those of the positive control were assessed to identify potential drug candidates (Table 2). Twenty compounds were predicted to be potential carcinogens, 9 compounds showed potential mutagenicity, and 25 compounds were predicted to be teratogenic.

DISCUSSION

Resistance to traditional anthelmintics (ivermectin, albendazole, and oxantel) has led to the need for alternative approaches, such as traditional medicines like Naaku Poochi Kolli Kudineer Chooranam (NPK), which has been shown to be effective against rodent pinworms [5] and *Haemonchus contortus* [6]. Our *in silico* analysis showed that the phytochemicals in NPK have a higher affinity than commercial medications when binding important parasite targets. According to Mottier et al. (2003), Cross et al. (1998), and Verhoeven et al. (1976), [20, 23, 24] the pharmacokinetic profile of benzimidazoles correlates with the lipophilicity (logP 1.5-4.5) of certain drugs, which guarantees efficient penetration into helminth cuticles by passive diffusion.

Notably, cucurbitacin E (-89.2 kcal/mol) and ellagic acid (-101.7 kcal/mol) from *P. kurroa* showed stronger binding to glutamate-gated chloride channels (GluCl) than ivermectin (-89.1 kcal/mol). Both compounds interact with Ser260, a critical residue for the allosteric modulation of ivermectin [26]. This suggests similar channel-opening effects through the stabilization of the open conformation of GluCl, leading to chloride influx and parasite paralysis. With beta-tubulin, albendazole possesses pan-isotype binding potential [27]. NPK compounds bind key albendazole interaction sites, Asn226, Gln11, and Cys12 [28, 29]. Additional residues, Ala97, Glu181, and Thr143, were identified as potential targets for stable drug interactions.

For nicotinic acetylcholine receptors (nAChR), NPK ligands outperform oxantel by binding to critical loops (B, C, D, F) in the orthosteric site [30]. Key interactions occurred with Lys212 (loop C) and Lys58 (between loops D/F), mirroring the agonism of ACR-16-like receptors [31]. This binding pattern may induce the characteristic loop C flip [23] that triggers muscular contractions, explaining NPK's observed expulsion effects of NPK [5]. The dual action of GluCl and nAChR suggests a mechanism in which initial ion influx-induced contractions are followed by hyperpolarization and paralysis.

Table 1: Binding energies of the highest scoring ten compounds from each plant having binding energy higher than the positive control

Plant	Compound	Target	Binding energy kcal/mol	Residues involved in Conventional H- bonds	Types of other bonds
Butea monosperma	Butein	Beta tubulin	-87.3	Gly98, Asn204, Glu69, Gln15	Hydrophobic-3
	Butein	nAChR	-115.7	B:Lys58, B:Asp190, A:Lys212, B:Asp81	Hydrophobic-1
	Laccijalaric ester II	nAChR	-113.1	B:Lys58, B:Asn77, A:Tyr117, A:Ser151	Hydrophobic-2
	Jalaric ester II	nAChR	-97.1	B:Lys58, B:Gln62, A:Asn118	Hydrophobic-4
Operculina turpethum	Luteolin	Beta tubulin	-89.4	Asn204, Gln15, Glu69	Hydrophobic-2
Pimpinella anisum	Luteolin	nAChR	-112.4	B:Asp190, B:Asp81, B:Glu193, A:Lys212, A: Lys 169	Hydrophobic-3 Coulombic-1
Nigella sativa	Silibinin	Beta tubulin	-135.9	Glu69, Ala97,Thr143, Gln11, Glu181, Asn226, Gln15	Hydrophobic-7 Coulombic- 1 Unfavorable-1
	Silibinin	nAChR	-113.5	B:Glu193, B: Gln62, B:Asp190, B:Lys58	Hydrophobic-1 Coulombic- 1
Foeniculum vulgare	Kaempferol	Beta tubulin	-114.8	Glu69, Gln15, Asn204, Gly 141	Hydrophobic-2
	Quercetin	Beta tubulin	-110	Glu181, Glu69, Gln11, Thr143	Hydrophobic-1
	Quercetin	nAChR	-110.8	B:Asp190, B:Asp81, B:Glu193, A:Lys212, A: Tyr 214, A:Lys169	Hydrophobic-3 Coulombic- 1 Unfavorable-1
	Xanthotoxol	Beta tubulin	-101.7	Ser138, Cys12	-
Picorrhiza kurroa	Ellagic acid	Beta tubulin	-95	Cys12, Gly98, Thr143, Glu69	Hydrophobic-1
	Ellagic acid	GluCl	-101.7	A: Ser 260, A: Asn 264, A:Asp 277, B: Gln 219	Hydrophobic-6
	Ellagic acid	nAChR	-117.1	B:Gln62, A:Asn118, A:Lys212	Coulombic- 2
	Cucurbitacin E	GluCl	-89.2	A: Gly 281, A:Ser 260	Hydrophobic-3 Unfavorable-1
Pimpinella anisum	Umbelliferone	Beta tubulin	-83.3	Gly140, Asn204, Gln15	Hydrophobic-3
Cassia augustifolia	Iso-rhamnetin	Beta tubulin	-86.8	Glu69	Hydrophobic-3
	Kaempferol	nAChR	-98.1	B:Glu193, B:Asp190, A:Lys212, A: Tyr 214, A:Lys169	Hydrophobic-3 Coulombic-1
	Aloe emodin anthrone	nAChR	-92.4	B:Lys58, A:Lys212, A:Tyr214, B:Asp190	Hydrophobic-1
Trachyspermum ammi	2-Methyl-3-glucosyloxy	Beta tubulin	-108.9	Glu69, Asn99	Unfavorable-1 -
	-5-isopropyl phenol	nAChR	-91	B:Glu193, B:Ser60, A:Lys212	-
Drug control					
Oxantel		nAChR	-47.7	B:Glu193, B:Ser192, B:Trp173, A:Tyr214	Hydrophobic-1
Ivermectin		GluCl	-89.1	A: Asn 264, B:Gln219, B: Leu218	Hydrophobic-12
Albendazole		Beta tubulin	-38.7	Asn226, Asn204, Gln11, Cys12	Hydrophobic-6

Table 2: ADMET properties of non-carcinogenic compounds with higher binding energies than drugs targeting GluCl, beta-tubulin, and nAChR

S. No	Name of the compound	Carcinogenicity	Ames mutagenicity	Developmental toxicity	Hepato-toxicity	Tox-Score
1.	Butein	Non-Carcinogen	Non-Mutagen	Toxic	TRUE	2
2.	Butin	Non-Carcinogen	Non-Mutagen	Toxic	TRUE	2
3.	2-Methyl-3-glucosyloxy-5-isopropyl phenol	Non-Carcinogen	Non-Mutagen	Toxic	TRUE	2
4.	Acetyl-eugenol	Non-Carcinogen	Non-Mutagen	Toxic	FALSE	1
5.	Acetovanillone	Non-Carcinogen	Non-Mutagen	Toxic	FALSE	1
6.	Isorhamnetin	Non-Carcinogen	Mutagen	Toxic	TRUE	3
7.	Kaempferol	Non-Carcinogen	Mutagen	Toxic	TRUE	3

8.	Luteolin	Non-Carcinogen	Non-Mutagen	Toxic	TRUE	2
9.	Nigellicine	Non-Carcinogen	Non-Mutagen	Toxic	TRUE	2
10.	Quercetin	Non-Carcinogen	Mutagen	Toxic	TRUE	3
11.	Silibinin	Non-Carcinogen	Non-Mutagen	Toxic	TRUE	2
P1	Ivermectin	Non-Carcinogen	Non-Mutagen	Non-Toxic	TRUE	1
P2	Albendazole	Non-Carcinogen	Non-Mutagen	Toxic	TRUE	2
P3	Oxantel	Non-Carcinogen	Non-Mutagen	Toxic	FALSE	1

Pharmacokinetic screening identified 21 compounds with optimal intestinal absorption and safety profiles including ellagic acid, luteolin, and silibinin. Eight candidates, namely butein, butin, 2-methyl-3-glucosyloxy-5-isopropyl phenol, acetyleugenol, acetovanillone, luteolin, nigellicine, and silibinin, combined superior target affinity with drug-like properties, making them prime candidates for *in vitro* and *in vivo* validation as next-generation anthelmintics. These findings substantiate NPK's traditional use while providing mechanistic insights for developing resistance-breaking formulations.

CONCLUSION

This computational study elucidated the potential anthelmintic mechanisms of the Siddha formulation NPK, demonstrating its ability to target crucial helminth receptors, glutamate-gated chloride channels, β-tubulin, and nicotinic acetylcholine receptors that mediate paralysis, egg expulsion, and parasite death. In addition, we identified several phytocompounds with favorable pharmacokinetic profiles as promising anthelmintic candidates. These findings provide a scientific basis for NPK's traditional use of NPK and highlight the need for experimental validation through *in vitro* and *in vivo* studies to confirm both the proposed mechanisms and the therapeutic potential of these bioactive compounds.

Conflict of interest

The authors declared no conflict of interest.

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