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HYPOURICEMIC ACTIVITY OF *MORINDA CITRIFOLIA* (NONI) BY INHIBITION OF XANTHINE OXIDASE FOR TREATMENT OF GOUT

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ABSTRACT

Gout is the most common form of inflammatory arthritis with a prevalence of 1–2% in developed countries. The major objectives in chronic gout management are to keep the serum uric acid level towards normal, prevent joint damage due to hyperuricemia and further occurrence as well as to promote the dissolution of existing uric acid crystals as well as prevent new crystal formation. The present work is aimed at *in-vitro* xanthine oxidase inhibitory activity between the enzyme xanthine oxidase isolated from bovine milk and *Morinda citrifolia* fruit extract and comparing against standard drug allopurinol. Further the activity would be ascertained by molecular docking studies of phytoconstituents identified in *Morinda citrifolia* fruit extract on xanthine oxidase obtained from protein data bank using Molegro virtual docker. The IC_{50} values were found to be 178.6µg/ml and 99.6 µg/ml for *Morinda citrifolia* fruit extract and allopurinol respectively. From the docking studies based on the MolDock score, Bisdemethylpinoresinol, Asperulosidic acid, Aucubin, Rutin and Americanin A were found to be the most effective phytoconstituents to bind with the selected xanthine oxidase enzyme as compared to the standard drugs. In conclusion, the extract of *Morinda citrifolia* was found to possess appreciable hypouricemic activity where one or more of the phytoconstituents present in the extract may be responsible for the activity. Further *in vivo* studies are required to confirm the hypouricemic activity of *Morinda citrifolia* fruit extract.

Key Words: Gout, xanthine oxidase, *Morinda citrifolia*, docking, arthritis

INTRODUCTION

Gout is the most common form of inflammatory arthritis. The prevalence is 0.12% as per International League of Nations against Rheumatic Diseases study in Bhigwan village of India. A study from Vellore revealed that 15.8% of the affected patients are less than 30 years of age. Urban Indian population is involved more than the rural population, with a prevalence of 1–2% in developed countries[1, 2]. They usually present in the form of podagra i.e. an acute onset of pain in joint, erythema and swelling of the first metatarsophalangeal joint. It is a disorder of purine metabolism associated with increased level of serum uric acid (serum uric acid >6. 8 mg/dL), crystallizes in the form of monosodium urate, deposit in joints, tendons and in the surrounding tissues[3, 4].

Gout affects about 1 to 2% of the Western population at some point in their lives. It has become more common in recent decades, which is believed to be due to increasing risk factors in the population, such as metabolic syndrome, longer life expectancy, and changes in diet. Gout is the common cause of arthritis in men aged over the fifty[5]. Incidence of gout in men is more than women because before menopause, estrogens promote urate wasting in the urine[6]. Gout was historically known as "the disease of kings" or "rich man's disease". It has been recognized at least since the time of the ancient Egyptians [7].

Treatment with non-steroidal anti-inflammatory drugs (NSAIDs), steroids, or colchicine improves symptoms. Once the acute attack subsides, levels of uric acid are usually lowered via lifestyle changes, and in those with frequent attacks, allopurinol or Probenecid provides long-term prevention. Taking vitamin C and eating a diet high in low fat dairy products may be preventative. These drugs have some side effects such as gastric ulcer, hypersensitivity; acute kidney injury and possibility of drug interaction with other prescribed drug such as erythromycin restrict their uses[8].The major objectives in chronic gout management are to keep the serum uric acid level towards normal, prevent joint damage due to hyperuricemia and further occurrence as well as to promote the dissolution of existing uric acid crystals as well as prevent new crystal formation[9]. Some non-pharmacological measures include restricted protein diet, life style modification, weight loss, low alcohol consumption and ensuring sufficient fluid intake[10].

Morinda citrifolia Linn belongs to the family Rubiaceae and habituated to Sub-Himalayan tracts, Darjeeling, Konkan and Andaman. The Indian Mulberry is commonly known as ashyuka, akshi & atchy in Ayurvedic & nunaa, togaru in siddha[11]. *Morinda citrifolia* (Noni) has been utilized for a considerable length of time to cure or counteract assortment of diseases by conventional therapeutic professionals in Hawaii and Polynesia. A complete review on *Morinda citrifolia* was

performed by Assi *et* al in 2016 which described the antimicrobial and antiseptic activity, antifungal activity, antioxidant activity, anti-inflammatory activity, anti-arthritic activity, anti-cancer activity, antidiabetic activity, wound healing activity, memory enhancing activity, anxiolytic and sedative activity, analgesic activity, gastric ulcer healing activity, antiemetic activity, gout and hyperuricemia healing activity, immunity enhancing activity, anti-viral activity, anti-parasitic activity, anti-tuberculosis activity, osteoporotic and otoscopic enhancer. These activities were carried out to the extent of *in-vitro*, *in-vivo* & clinical trial stages[12].

The present work is aimed at *in-vitro* and *in-silico docking* studies between the enzyme xanthine oxidase isolated from bovine milk and the phytoconstituents identified in *Morinda citrifolia* fruit extract and comparing the inhibitory activity against standard drug allopurinol.

MATERIALS AND METHODS

Plant Extract

The full spectrum standardized extract of *Morinda citrifolia* fruit was obtained from Amsar Goa Pvt Ltd, Goa. The plant was authenticated by Dr Laxmi Morajkar, Head Ayurveda Division (Voucher specimen Number: AGPL/039/13-14). *Morinda citrifolia* fruits were shade dried and kept in an air tight container. Extraction was carried out with water and ethanol in a ratio of 80:20 by a simple maceration technique. The hydro alcoholic solvent of 1 liter was added to the shade dried plant powder of 100 g and placed on a mechanical shaker for a period of 4 hours. Then the solution is filtered through Whatmann No.1 filter paper. The filtrate concentrated using flash evaporator and further processed to dryness in a vacuum desiccator. 4.5 g is the percentage yield of the hydroalcholic extract of *Morinda citrifolia*.

In-vitro xanthine oxidase inhibitory activity

The enzyme xanthine oxidase was prepared and isolated from Bovine's milk as proposed by Jun Ichi Toyama et al[13]. Xanthine oxidase catalyzes the oxidation of hypoxanthine and xanthine to uric acid. Usually, the enzyme is isolated from bovine milk. The enzyme is inhibited by allopurinol and related compounds. The production of uric acid from the substrate (xanthine) can be determined by measuring the change in optical density in the UV range. The plant extract is incubated with xanthine oxidase (Isolated from bovine milk), EDTA and phosphate buffer solution (pH 7.8) at 37°C. Control solutions without test compound are incubated under identical conditions. Following addition of xanthine, the change in absorbance is determined at a wavelength: 290 nm, line path: 10 mm & final volume: 1.0 ml. The percent inhibition of xanthine oxidase is determined relative to control solutions. IC_{50} values of plant extract and Standard Drug Allopurinol was calculated at a concentration of 50-800 µg/ml[14-16]. The percentage inhibition was calculated by,

$$\text{Percentage inhibition} = (V_c - V_t / V_c) \times 100$$

Where V_c = Absorbance of control, V_t = Absorbance of test

Molecular docking study

Preparation of Ligand

The 3D structures of the phytoconstituents of *Morinda citrifolia* are obtained from Pubchem compound database[17, 18]. The ligands are imported to the workspace and preparation of them is done. The docking scores of the active constituents are compared against the standard drugs (Allopurinol, Febuxostat) obtained from the drug bank in mol format[19].

Preparation of Enzyme

The target for docking studies is selected as Xanthine oxidase. Docking analysis is done by initially selecting the target for the disease and followed by obtaining the 3D structure of Xanthine oxidase (3EUB) from protein data bank in .pdb format. Aldehyde oxidase is a xanthine oxidase related enzyme with emerging importance due to its role in the metabolism of drugs and xenobiotics. The enzyme is the newest of its kind as it has been released in 2015. It is an oxidoreductase containing the molecule aldehyde oxidase with chain A (length 1338) [20]. The PDB file format cannot accommodate bond order information due to deprived or misplaced assignments of unequivocal hydrogen. Thus, MVD analyzer was used for assigning appropriate bond orders, bonds, charges and its hybridization. The possible binding site of the target was calculated by using algorithm (cavity detection). The simulations search space was exploited around the active side cleft 15.0 Angstroms. Docking analysis was done by opt the Molegro Virtual Docker (MVD) software. It gives the confirmation on ligand dock to target and widely preferred by medicinal chemists [21]. The Mol-Dock score was worked on Piecewise Linear Potential (PLP), where the structure of target-ligand and its docking score function parameters are fit42. In an advance GEMDOCK were extended with furthermore new H-bond and its charges [22-24].

MolDock Optimizer

In MVD, the differential evolution algorithm was guided by the elected parameters includes number of runs is 5, population size is 50, maximum interactions are 2000, crossover rate is 0.9 and scaling factor is 0.5. Pose clustering was preferred to make certain appropriate binding mode in the selected cavity.

MolDock score

Select the ignore-distant-atoms to disregard the atoms which are far from docking site. Furthermore, check the H-bond direction between the impending donors and acceptors. Option the cavity with a radius of 25 Å in the binding site of the target made in X, Y and Z directions.

Re rank Score

Re rank scoring functions are used to generate and predict the models for evaluating the chemical properties (e.g. QSAR). The re rank scoring function was computationally expensive compare to the scoring function in docking simulation. In general, it was most preferable in finding the best pose in among the poses originates from the same ligand. Although the re rank score in MVD gives an approximate to the strength of the interaction, the chemical units are not calibrated and do not consider the complex contributions (entropy) in the account. The re rank score accurately rank the dissimilar poses of individual ligands. The measuring of binding affinity was used subsequently to get a rough estimate of the highest ranked poses.

Lipinski Rule

The medicinal chemist Christopher Lipinski and his colleagues analysed the physicochemical properties of more than 2000 drugs and candidate drugs in clinical trials and concluded that a compound is more likely to be membrane permeable and easily absorbed by the body, if it matches the rule[25]. Lipinski rule was applied to 4 standard drugs and 19 of the phytoconstituents identified in *Morinda citrifolia* fruit extract.

Table 1: *In-Vitro* xanthine oxidase inhibitory Activity of *Morinda citrifolia* fruit extract

Concentration (µg/ mL)	Log concentration	% inhibition	
		Allopurinol	***Morinda citrifolia***
50	1.699	52.23±0.28	28.90±1.26
100	2	49.44±0.14	21.26±0.89
200	2.301	49.75±1.28	19.35±1.16
400	2.602	48.64±2.34	18.76±1.17
800	2.903	47.98±0.47	16.54±3.25
***In-vitro* activity was carried out in triplicate and the data was expressed as mean ± standard error mean (S.E.M.)**			

Table 2: Ranking of poses & ligands (Phytoconstituents) based on MolDock Score docked by Molegro virtual docker on Xanthine oxidase (PDB ID: 3EUB)

Name	Ligand	MolDock Score	Re rank Score	H Bond
[00]bisdemethylpinoresinol	Bisdemethylpinoresinol	-155.773	-124.883	-12.0215
[00]asperulosidic acid	Asperulosidic Acid	-140.66	-97.1321	-12.1074
[00]aucubin	Aucubin	-120.27	-65.256	-14.8219
[00]Rutin	Rutin	-116.058	-80.1913	-13.0685
[00]Americanin A	Americanin A	-115.121	-95.0757	-8.00946
[00]deacetylasperulosidic acid	Deacetylasperulosidic Acid	-107.564	-91.7448	-10.5578
[00]Febuxostat	**Febuxostat**	**-106.411**	**-88.3713**	**-2.90712**
[00]catechin	Catechin	-104.844	-88.4001	-9.14073
[00]ursolic acid	Ursolic Acid	-104.82	-41.4987	-4.32073
[00]epicatechin	Epicatechin	-99.6627	-87.5459	-9.91361
[00]damnacanthal	Damnacanthal	-92.1703	-81.8277	-5.79694
[00]glucuronic acid	Glucuronic Acid	-86.0149	-76.2423	-19.8964
[00]arabinose	Arabinose	-83.6722	-73.8732	-21.4554
[00]Tisopurine	Tisopurine	-81.9948	-68.1857	-6.12277
[00]Oxypurinol	Oxypurinol	-81.2059	-66.8756	-4.68465
[00]morindone	Morindone	-80.4199	-78.1986	-5.86909
[00]Allopurinol	**Allopurinol**	**-80.4094**	**-65.5767**	**-13.7011**
[00]limonene	Limonene	-77.5669	-63.7917	0
[00]scopalamine	Scopalamine	-73.6561	-57.0856	-2.18873
[00]alizarin	Alizarin	-73.2059	-69.6957	-5.07506
[00]rhamnose	Rhamnose	-71.8635	-69.1494	-15.7587
[00]galactose	Galactose	-71.7472	-69.1742	-15.104
[00]butylhydroxytoluene	Butylhydroxytoluene	-70.3288	-63.3454	0

Table 3: Ranking of poses & ligands (Phytoconstituents) based on H Bond Score docked by Molegro virtual docker on Xanthine oxidase (PDB ID: 3EUB)

Name	Ligand	MolDock Score	Re rank Score	H Bond
[00]arabinose	Arabinose	-83.6722	-73.8732	-21.4554
[00]glucuronic acid	Glucuronic Acid	-86.0149	-76.2423	-19.8964
[00]rhamnose	Rhamnose	-71.8635	-69.1494	-15.7587
[00]galactose	Galactose	-71.7472	-69.1742	-15.104
[00]aucubin	Aucubin	-120.27	-65.256	-14.8219
[00]Allopurinol	Allopurinol	-80.4094	-65.5767	-13.7011
[00]Rutin	Rutin	-116.058	-80.1913	-13.0685
[00]asperulosidic acid	Asperulosidic Acid	-140.66	-97.1321	-12.1074
[00]bisdemethylpinoresinol	Bisdemethylpinoresinol	-155.773	-124.883	-12.0215
[00]deacetylasperulosidic acid	Deacetylasperulosidic Acid	-107.564	-91.7448	-10.5578
[00]epicatechin	Epicatechin	-99.6627	-87.5459	-9.91361
[00]catechin	Catechin	-104.844	-88.4001	-9.14073
[00]Americanin A	Americanin A	-115.121	-95.0757	-8.00946
[00]Tisopurine	Tisopurine	-81.9948	-68.1857	-6.12277
[00]morindone	Morindone	-80.4199	-78.1986	-5.86909
[00]damnacanthal	Damnacanthal	-92.1703	-81.8277	-5.79694
[00]alizarin	Alizarin	-73.2059	-69.6957	-5.07506
[00]Oxypurinol	Oxypurinol	-81.2059	-66.8756	-4.68465
[00]ursolic acid	Ursolic Acid	-104.82	-41.4987	-4.32073
[00]Febuxostat	Febuxostat	-106.411	-88.3713	-2.90712
[00]scopalamine	Scopalamine	-73.6561	-57.0856	-2.18873
[00]butylhydroxytoluene	Butylhydroxytoluene	-70.3288	-63.3454	0
[00]limonene	Limonene	-77.5669	-63.7917	0

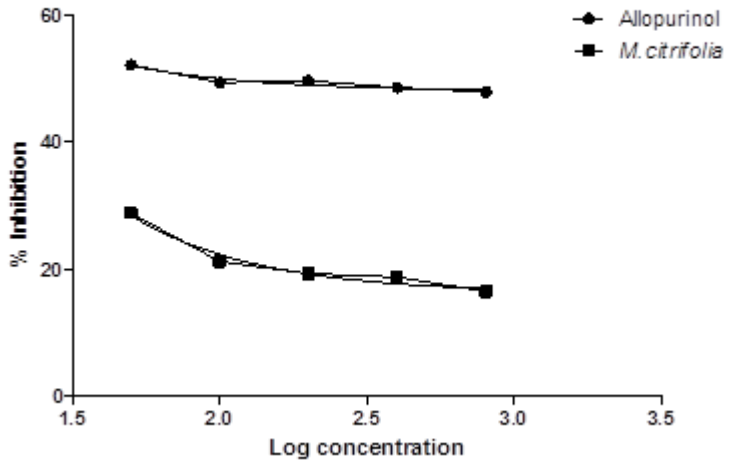
Table 4: Target amino acid binding residues of phytoconstituents and standard drugs on xanthine oxidase evaluated using ligand energy inspector tool

Ligand	Target Atoms	Binding amino acid residues
Febuxostat	3EUB [A]	Ala 262, Asn 268, Gln 261, Glu 409, Gly 267, Gly 356, Gly 357, Ile 265, Ile 360, Leu 405, Lys 256, Lys 406, Met 266, Pro 260, Pro 263, Pro 407, Ser 270, Ser 354, Ser 361, Thr 269, Val 264, Val 271
Allopurinol	3EUB [A]	Ala 353, Asn 268, Gly 267, Gly 356, Gly 357, His 358, Ile 265, Met 266, Met 352, Ser 270, Ser 354, Ser 361, Thr 269, Val 264, Val 271
Bis demethyl pinoresinol	3EUB [A]	Ala 353, Asn 268, Asp 365, Asp 367, Gly 267, Gly 357, His 358, His 363, Ile 265, Ile 349, Leu 312, Leu 344, Leu 438, Met 266, Met 352, Ser 270,Ser 354, Ser 361, Ser 366, Thr 269, Val 271
Americanin A	3EUB [A]	Ala 353, Asn 268, Asp 404, Glu 409, Gly 267, Gly 356, Gly 357, His 358, Ile 265, Ile 360, Ile 410, Leu 405, Lys 256, Lys 406, Met 266, Pro 263, Pro 407, Ser 270, Ser 354, Ser 361, Thr 269, Val 264, Val 271
Aucubin	3EUB [A]	Ala 308, Ala 353, Asn 268, Gly 267, Gly 309, Gly 356 Gly 357, His 358, Ile 265, Ile 360, Ile 410, Leu 294, Leu 355 Leu 405, Leu 411, Met 266, Pro 263, Ser 270, Ser 354, Ser 361, Thr 269, Val 264, Val 271
Asperulosidic Acid	3EUB [A]	Ala 262, Arg 362, Asn 268, Gln 261, Glu 274 Gly 267, Gly 356, Gly 357, His 282, His 358, Ile 265 Ile 360, Lys 256, Lys 399, Met 266, Pro 260, Pro 263, Ser 270, Ser 361, Val 264, Val 271

Table 5: Evaluation of Ligands (Phytoconstituents) based on Lipinski Rule

Sr. No.	Ligand (Phytoconstituents)	Molecular weight in Daltons	Log P	Hydrogen bond acceptor	Hydrogen bond donor
1	Alizarin	240.04	1.057	4	2
2	Americanin A	328.09	0	6	3
3	Arabinose	150.05	-2.653	5	4
4	Asperulosidic acid	432.13	-2.41	12	6
5	Aucubin	346.13	-2.39	9	6
6	Bisdemethylpinoresinol	330.11	1.532	6	4
7	Butylhydroxytoluene	220.18	4.46	1	1
8	Catechin	290.08	0.852	6	5
9	Damnacanthal	282.05	0.992	5	1
10	Deacetylasperulosidic acid	390.12	-3.15	11	7
11	Epicatechin	290.08	0.852	6	5
12	Galactose	180.06	-1.697	6	5
13	Glucuronic acid	194.04	-1.54	7	5
14	Limonene	136.13	3.729	0	0
15	Morindone	270.05	0.735	5	3
16	Rhamnose	164.07	-0.994	5	4
17	Rutin	610.15	-0.735	16	10
18	Scopalamine	303.15	0.59	5	1
19	Ursolic acid	456.36	8.954	3	2
20	Allopurinol	136.04	-0.64	5	2
21	Febuxostat	316.09	1.628	5	1
22	Oxypurinol	152.03	-0.865	6	3
23	Tisopurine	152.02	-0.742	4	2

Figure 1: *In vitro* xanthine oxidase inhibitory activity of *Morinda citrifolia* fruit extract



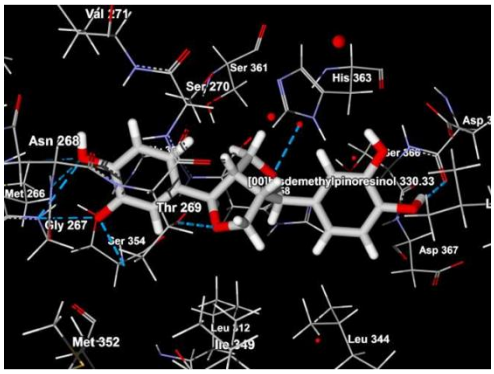


Figure 2: Docked view of [00] Allopurinol

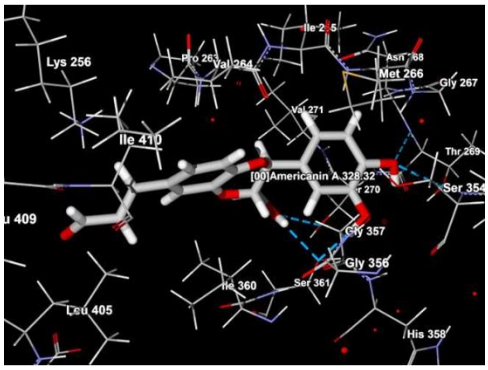


Figure 3: Docked view of [00] Febuxostat

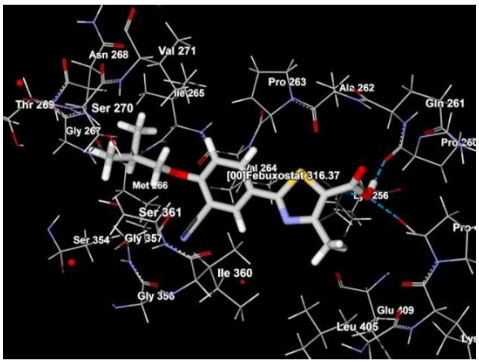


Figure 4: Docked view of [00] Americanin A

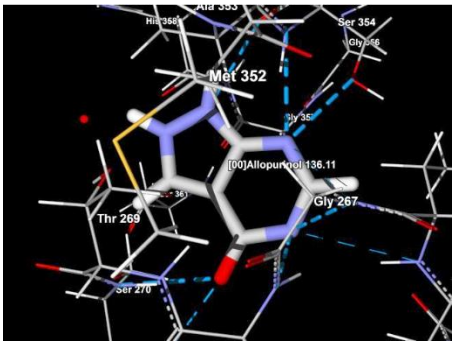


Figure 5: Docked view of [00] Bisdemethylpinoresinol

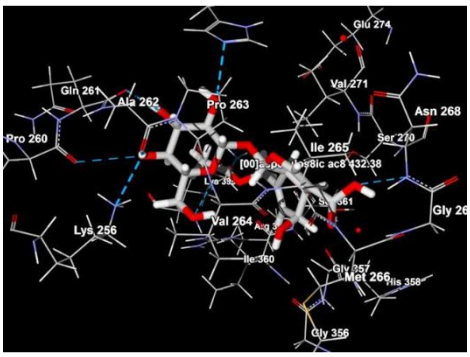


Figure 6: Docked view of [00] Asperulosidic Acid

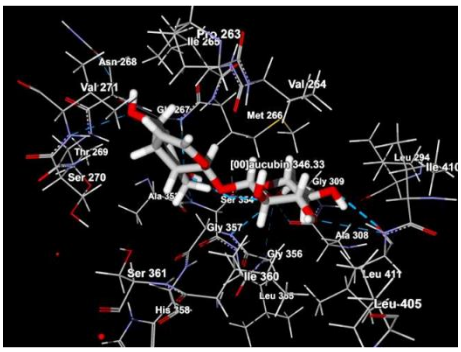


Figure 7: Docked view of [00] Aucubin

Statistical analysis

In-vitro activity was carried out in triplicate and the data was expressed as mean ± standard error mean (S.E.M.). The inhibitory concentration of xanthine oxidase by 50%, (IC_{50}) was graphically evaluated by a linear regression method using Graph Pad Prism (Ver. 5.0) software.

RESULTS AND DISCUSSION

In-vitro xanthine oxidase inhibitory activity

Morinda citrifolia extract showed greater absorbance than allopurinol at similar concentrations indicating the extract has less xanthine oxidase inhibitory activity than the standard allopurinol and it is represented in Table 1 & Figure 1. The IC_{50} values were found to be 178.6µg/ml and 99.6 µg/ml for *Morinda citrifolia* fruit extract and allopurinol and respectively.

In- silico docking analysis

From the docking studies based on the MolDock score, Bisdemethylpinoresinol, Asperulosidic acid, Aucubin, Rutin and Americanin A were found to be the most effective ligands to bind with the selected xanthine oxidase. All of these compounds have been reported to have appreciable anti-inflammatory activity as represented in tables 2 & 3. Bisdemethylpinoresinol had the highest rank according to the MolDock score and re rank score with values of -155.773 and -124.883 respectively. However, its H bond score is only -12.0215 which was ranked at 9th position. This proves the compound is better than the standard drugs like allopurinol and febuxostat in its affinity to the protein. Asperulosidic acid was second in rank according to MolDock score and re rank score with values of -140.66 and -97.1321. It was ranked 8th according to the H bond score with a value of -12.1074. This compound too has better affinity than the standard drugs. Aucubin ranked third with a MolDock score of -120.27. However, its re rank score is only -14.8219 which is

less than even allopurinol and febuxostat which are standard drugs. The MolDock score and the H bond score is higher than that of the standards but the re rank score is less than the standards. Fourth in rank according to MolDock score is rutin with a value of -116.058. Its re rank score is -80.1913 and H bond score is -13.0685. Rutin has a better MolDock score than the standard drugs. Its re rank score is less than that of febuxostat (-88.3713) and higher than allopurinol (-65.5767). The H bond score pattern was vice versa with a score less than that of allopurinol (-13.7011) and higher than febuxostat (-2.90712). Americanin A is ranked fifth according to MolDock score of -115.121 and third in re rank with a score of -95.0757. It has a better MolDock and re rank score than the standards, however, it has an H bond score of only -8.00946 which is less than the standard allopurinol.

Docking pattern

The docked images show the amino acids of chain A of the protein that are involved in interaction with the phytoconstituents and the standard drugs (Figures 2-7). The various amino acid residues involved in interaction for each of allopurinol, febuxostat, bisdemethylpinoresinol, asperulosidic acid, Americanin A and aucubin are given in table 4. The variation in the residues involved explains the difference in affinity of the various ligands towards the enzyme. In most cases, similar residues are present around the binding cavity indicating these ligands bind to the same cavity.

Lipinski Rule

From the results of the Lipinski rule (Table 5), 5 of the 23 ligands deviated from the rule. These include asperulosidic acid, aucubin, rutin, ursolic acid and deacetylasperulosidic acid. This excludes 3 of the 5 chosen ligands (based on MolDock score) i.e. asperulosidic acid, aucubin and rutin. Bisdemethylpinoresinol and Americanin A complied well with the rule and had values for the various parameters within the range. The standard drugs, allopurinol and febuxostat showed good compliance with the rule.

CONCLUSION

The present study investigated *in vitro* and *in silico* anti-uroliathitic activity of *Morinda citrifolia*. *In silico* docking was performed for 23 compounds which include 19 phytoconstituents reported in *Morinda citrifolia* and 4 standard drugs for the treatment of gout. The ligands were obtained from PubChem compound database and the protein was obtained from protein data bank. Flexible docking was performed using Molegro virtual docker. MolDock score, Re rank score and H bond interactions were used as parameters for evaluation of the protein-ligand complexes.

In vitro activity was performed using xanthine oxidase isolated from bovine milk by following standard procedures and the standardised extract of *Morinda citrifolia* was obtained from Amsar Goa pvt.ltd. The IC_{50} value for the extract at 50-800 µg/ml was established by plotting the graph with log concentration on x axis and percentage inhibition on y axis. The test extract was compared with the standard drug allopurinol and it showed least inhibition as compared to that of the standard drug. In conclusion, the extract of *Morinda citrifolia* was found to possess appreciable hypouricemic activity where one or more of the phytoconstituents present in the extract may be responsible for the activity. Further *in vivo* studies are required to confirm the hypouricemic activity of *Morinda citrifolia* extract.

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REFERENCES

1. Chopra A, Patil J, Billempelly V, Relwani J, Tandle HS. Prevalence of rheumatic diseases in a rural population in western India: a WHO-ILAR COPCORD Study. J Assoc Phys India 2001; 49:240-6.
2. Doherty M. New insights into the epidemiology of gout. Rheumatology 2009; 48(2):2-8.
3. Hamburger M, Baraf HS, Adamson TC, Basile J, Bass L, Cole B et al. recommendations for the diagnosis and management of gout and hyperuricemia. Postgraduate medicine 2011; 123 Suppl1:3-6.
4. Thiele RG, Schlesinger N. Diagnosis of gout by ultrasound. Rheumatology 2007; 46(7):1116-21.
5. Helmick CG, Felson DT, Lawrence RC, Gabriel S, Hirsch R, Kwoh CK et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Arthritis Rheum 2008; 58(1):15-25.
6. Wallace KL, Riedel AA, Joseph-Ridge N, Wortmann R. Increasing prevalence of gout and hyperuricemia over 10 years among older adults in a managed care population. J Rheumatol 2004; 31(8):1582-7.
7. Dirken-Heukensfeldt KJ, Teunissen TA, Van de Lisdonk EH, Lagro-Janssen AL. "Clinical features of women with gout arthritis." A systematic review. Clin. Rheumatol 2010; 29(6):575-82.
8. Chen HW, Chen KC, Chen JS. Colchicine and NSAID combination causing acute kidney injury. J Coll Phys Surg Pak 2012; 22:737-9.
9. Roddy E. Revisiting the pathogenesis of podagra: why does gout target the foot? J Foot Ankle Res. 2011 May 13; 4(1):13.
10. Underwood M. Diagnosis and management of gout. British Medical Journal 2006; 332(7553):1315.
11. Khare CP. Indian medicinal plants: an illustrated dictionary. Springer Science & Business Media, 2008.
12. Assi RA, Darwis Y, Abdulbaqi IM, Vuanghao L, Laghari MH. *Morinda citrifolia* (Noni): A comprehensive review on its industrial uses, pharmacological activities, and clinical trials. Arabian J. Chem 2015; doi.org/10.1016 /j.arabjc.2015.06.018
13. Toyama JI. A simple method for the isolation of xanthine oxidase from milk. The Journal of Biochemistry 1933; 17(1):131-3.
14. Heinz F, Reckel S. Xanthine oxidase In: Bergmeyer HU (ed) Methods of Enzymatic Analysis. Vol. III, 3rd edn. Verlag Chemie Weinheim, Deerfield Beach, Basel. 1983:211-6.
15. Rhoden E, Telöken C, Lucas M, Rhoden C, Mauri M, Zettler C et al. Protective effect of allopurinol in the renal ischemia–reperfusion in uninephrectomized rats. General Pharmacology: The Vascular System 2000; 35(4):189-93.
16. Shinosaki T, Yonetani Y. Hyperuricemia induced by the uricosuric drug probenecid in rats. Jpn. J. Pharmacol 1991; 55(4):461-8.
17. Bolton EE, Wang Y, Thiessen PA, Bryant SH. PubChem: integrated platform of small molecules and biological activities. Annu Rep Comput Chem 2008; 4: 217-41.
18. Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, Tzur D, Gautam B, Hassanali M. DrugBank: a knowledgebase for drugs, drug actions and drug targets. Nucleic Acids Res 2008; 36 Suppl 1:D901-6.

19. Pauff JM, Cao H, Hille R. Substrate orientation and catalysis at the molybdenum site in xanthine oxidase crystal structures in complex with xanthine and lumazine. Journal of Biological Chemistry 2009; 284(13):8760-7.
20. Bernstein FC, Koetzle TF, Williams GJ, Meyer EF, Brice MD, Rodgers JR, Kennard O, Shimanouchi T, Tasumi M. The protein data bank. Eur J Biochem 1977; 80(2):319-24.
21. Thomsen R, Christensen MH. MolDock: a new technique for high-accuracy molecular docking. J Med Chem 2006; 49(11):3315-21.
22. Yang JM, Chen CC. GEMDOCK: a generic evolutionary method for molecular docking. Proteins: Structure, Function, and Bioinformatics 2004; 55(2):288-304.
23. Gehlhaar D, Bouzida D, Rejto P. Fully automated and rapid flexible docking of inhibitors covalently bound to serine proteases. In Evolutionary Programming VII, Springer Berlin/Heidelberg, 1998.
24. Gehlhaar DK, Verkhivker G, Freer S. Docking conformationally flexible small molecules into a protein-binding site through simulated evolution. In abstracts of papers of the american chemical society 1995.
25. Lipinski CA. Lead-and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies 2004; 1(4):337-41.

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