Current Trends in **Pharmaceutical Research** 2018, 5(1):1-23
ISSN 2310-4820
 www.dibru.ac.in/ctpr

Pharmacological screening of *Centella asiatica* for its anti-amoebic properties: An *in-silico* approach

Birupakshya Paul Choudhury[1*], Monjur Ahmed Laskar[1], Manabendra Dutta Choudhury[1,2]

[1] Bioinformatics Centre, Assam University, Silchar, Assam, India

[2] Department of Life & Bioinformatics, Assam University, Silchar, Assam, India

Abstract

Centella asiatica, or Indian pennywort is a herb and is well-known for its medicinal properties according to ancient literature and scientific reports. Infection of Entamoeba histolytica results in amoebic dysentery, amoebic liver abscess and is one of the leading cause of mortality worldwide. Amoebapore proteins are one of the key virulence factors of E. histolytica which have been found to be involved in the pathogenesis of liver abscess. The objective of the study was to identify the anti-amoebic properties of the phytochemicals present in C. asiatica and evaluate the drug likeliness of the compounds. As per literature survey, 46 compounds were recognized from C. asiatica. ADMETox screening was performed using Mobyle RPBS. The structure of Amoebapore was adopted from Protein Data Bank (PDB ID- 1OF9). Molecular docking was performed using FlexX. Drug likeliness of the screened compounds was evaluated using Molsoft L.L.C. Out of 46 compounds, 21 were able to dock and few amongst these exhibited strong affinity towards the target as compared to control. Quercetin presented greatest binding affinity to Amoebapore. These compounds also obeyed the Lipinski's rule and passed ADMETox screening. Stronger binding of certain phytochemicals to the target indicates better medicinal properties against the target protein. Also, the compounds abide by Lipinski's rules, thus revealing drug like properties. Thus, quercetin may be considered as an effective anti-amoebic agent.

Keywords: *Entamoeba histolytica,* amoebic liver abscess, amoebapore, molecular docking, herbal drug.

*Current Address: Department of Zoology, University of Delhi, Delhi 110007; Email- birupakshyapc@gmail.com

1. Introduction

There is an increasing trend of using natural products for remedial purposes. As per reports, 80% of the population depends on herbal medicine (Ekor, 2013). *Centella asiatica* or Indian Pennywort also known as Gotu Kola, Mandukaparni, Jalbrahmi or Thankuni belonging to the *Umbelliferae (Apiceae)* family is a perennial herbaceous creeper with soft, slender green stalk and round leaves (Gohil *et al* 2010; Sushen *et al* 2017). The plant bears light purple to pink or white flowers and oval fruits. It grows in tropical, swampy areas widely distributed in and around Asia (Gohil *et al* 2010). *Centella asiatica* (CA) has been used since ancient times both in Ayurvedic (Babu *et al* 1995) and Chinese traditional systems (Gohil *et al* 2010). This plant has also been used by the traditional healers in some parts of the continent including Bangladesh (Kadir *et al* 2014). CA extract has been used in traditional wound healing and these properties have been validated through *in vivo* studies (Gohil *et al* 2010). CA has also been used as a nerve tonic since the ancient time and is well known as a neuroprotective agent (Sushen *et al* 2017). CA extracts possess memory enhancing properties apart from being effective in Alzheimer's disease (AD) (Gohil *et al* 2010). These herbs are also used in case of venous insufficiency (Gohil *et al* 2010), piles (Devi Prasad *et al* 2013) and other bowel disorders. CA also possess antimicrobial and antifungal (Jagtap *et al* 2009); anti-ageing and antioxidant (Pittella *et al* 2009); antidepressant, antiepileptic and sedative (Gohil *et al* 2010); anxiolytic (Wanasuntronwong *et al* 2012); anti-inflammatory, anticancer, anti-diabetic, cardio-protective and radio-protective properties (Sushen *et al* 2017). There has been some reports regarding the anti-amoebic properties of CA. The herb consists of saponins, tanins, free amino acids, flavonoids, sterols, essential acids with major part being the saponins (Gohil *et al* 2010).

Entamoeba histolytica is a protozoal parasite belonging to the family *Entamoebidae*. *E. histolytica* has been reported to possess cytolytic properties as a result of which it disrupts the intestinal mucosa, penetrates host tissue thereby causing ulcer, amoebic dysentery, amoebic liver abscess (ALA) and other related disorders (William 2008). Amoebiasis and associated disorders are the third leading cause of mortality worldwide (Davis *et al* 2017). The key virulence factors identified till date are the *Gal/GalNAc lectin* which mediates adhesion to host cells (William 2008), *Amoebapores* that produce pores in the host cells (Lynch *et al* 1982; William 2008) and *Cysteine Proteases* which are responsible for tissue invasion (William 2008; Ralston and William 2011). Amoebapores are family of small channel forming peptides present in the cytoplasmic vesicles of the trophozoites that have maximum activity in acidic pH (Ralston and William 2011). Three isoforms of amoebapores have been reported- A, B and C occurring in the ratio 35:10:1 (Leippe 1997; William 2008) respectively. But according to few reports the ratio of the amoebapore A, B and C is 29:9:1 (Bracha *et al* 2002). Amoebapores kill human Jurkat T cells and produce pores on host cells (Andrä 2004). It has been found that inhibition of amoebapore gene expression leads to the loss of virulence in *E. histolytica* accompanied by a reduced occurrence of ALA *in vivo*, thus suggesting it to be a key factor in tissue invasion (Bracha *et al* 2002). Amoebapore type A consists of 77 residues with 5 alpha helices and the structure has been termed as folded leaf structure (Grotzinger *et al* 2004).

The traditional healers used CA for treating stomach disorders (Kadir *et al* 2014), which may also include amoebiasis. Thus, the present *in-silico* study aims at evaluating the antiamoebic potential of the phytochemicals present in CA. Since amoebapores are one of key pathogenic factor involved in host tissue penetration, it has been considered as the target for the present study to assess the anti-amoebic activity of CA. This study is the first of its kind that reports the putative amoebapore inhibitory components in CA. This herb is easily available and hence may prove useful in designing novel drugs against *E. histolytica* and other disease causing agents against which CA has been reported to be effective.

2. Materials and Methods

2.1 Selection of Phytochemicals and Inhibitors:

Literature were searched for obtaining information on the biologically active phytochemicals of CA. Binding Database was searched for selecting conventional protozoal inhibitors which represented the control group for this study. ACD/ChemSketch and NCBI-Pubchem database was used to draw the structure and determine *Simplified Molecular-Input Line-Entry (SMILE)* notation of the compounds and the inhibitors.

2.2 Target selection: Amoebapore proteins have been found to have a major role in the pathogenesis of *E. histolytica* but there was no evidence relating the effect of CA on this protein. Keywords involving the combination of words '*Centella* *asiatica*', '*Entamoeba histolytica*' and 'amoebapore' were searched in NCBI-PubMed but no results were found. Hence, amoebapore A was considered as the target for the present study. Structure of amoebapore A (Grotzinger *et al* 2004) was adopted from RCSB-Protein Data Bank (PDB). The PDB-ID of amoebapore A is 1OF9. (Fig.1).

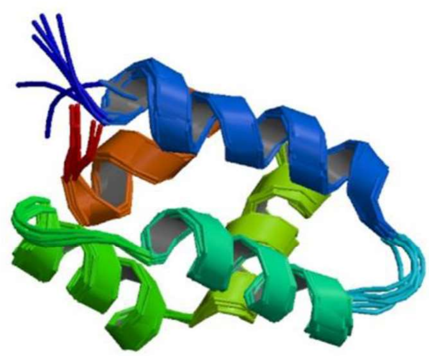


Fig1: Structure of Amoebapore A. Source: RCSB-Protein Data Bank (PDB). The PDB-ID of amoebapore A is **1OF9**.

2.3 Absorption Distribution Metabolism Excretion Toxicity (ADME-Tox) filtering:

The *ADME-Tox* analyses of the phytochemicals was conducted using Mobyle RPBS server. This analysis was done to find out if the compounds had the ability to act as drug or drug like molecules. Basically, it was evaluated whether the compounds abide by the Lipinski's rule of five and few other parameters which add to the absorption, distribution, metabolism, excretion and toxicity properties of the compound under test. The SMILE notations of the phytochemicals were collected from NCBI-Pubchem database. The SMILEs were converted to SDF format using Open Babel software. The phytochemicals were uploaded in the Mobyle RPBS server in the SDF format.

2.4 Molecular Docking:

Molecular docking was performed according to Chowdhury *et al*, 2012. Docking of the phytochemicals as well as inhibitors with the target protein was performed separately to determine their binding affinity with the target. The docking score of the phytochemicals and inhibitors with that of the target was recorded. The phytochemicals/inhibitors and target protein were in SDF and PDB format respectively. Docking was done using FlexX software.

2.5 Drug-Likeness and molecular property prediction:

Drug likeliness of those phytochemicals were evaluated which displayed strong affinity towards the target. The SMILE notations of those compounds were submitted to Molsoft L.L.C server for this analysis.

3. Results:

3.1 Phytochemicals and inhibitors:

46 biologically active phytochemicals were identified from literature which includes 1,5-di-O-caffeoyl quinic acid, 3,4-di-ocaffeoyl quinic acid, 3,5-Di-O-caffeoyl quinic acid and 4,5-di-O-caffeoyl quinic acid (Satake *et al* 2007); (20R)-ginsenoside Rg3 and (20S)-ginsenoside Rg3 (Weng *et al* 2011); 3-epimaslinic acid (Yoshida *et al* 2005); Apigenin (Bhandari *et al* 2007); Asiatic acid (Yoshida *et al* 2005; Rafamantanana *et al* 2009); Asiaticoside (Rafamantanana *et al* 2009); Asiaticoside F and Asiaticoside G (Nhiem *et al* 2011); Bayogenin (Orhan *et al* 2012); Brahminoside (Orhan *et al* 2012); Cadiyenol (Govindan *et al* 2007); Campesterol (Gohil *et al* 2010); Castilliferol (Satake *et al* 2007); Castillicetin (Orhan *et al* 2012); Centellasaponins B, C and D (Matsuda *et al* 2001); Chlorogenic acid (Satake *et al* 2007); Corosolic acid (Yoshida *et al* 2005); D-gulonic acid and Docosyl ferulate (Yu *et al* 2007); Ginsenoside Mc, Rk1, Rg5 and Y (Weng *et al* 2011), Irbic acid, Isochlorogenic acid (Orhan *et al* 2012); Kaempferol and Kaempferol-3-O-ß-D-glucoside (Satake *et al* 2007); Madasiatic acid, Madecassic acid, Madecassoside and Myricetin (Orhan *et al* 2012); Pomolic acid (Yoshida *et al* 2005); Quadranoside IV (Nhiem *et al* 2011); Quercetin (Satake *et al* 2007; Bhandari *et al* 2007); Rutin (Bhandari *et al* 2007); Rosmarinic acid (Yoshida *et al* 2005); Sceffoleoside (James and Dubery 2009); Stigmasterol (Gohil *et al* 2010); Ursolic acid (Orhan *et al* 2012) and Vitamin C (Singh *et al* 2011).

As of the inhibitors, 25 known protozoal inhibitors have been taken from Binding database whose IDs are: BDBM50331776, BDBM19518, BDBM50331771, BDBM50331786, BDBM50331779, BDBM50331772, BDBM50331775, BDBM50331788, BDBM50331783, BDBM50331782, BDBM50331774

(Beaulieu *et al* 2010); BDBM50229129 (Chen *et al* 2008); BDBM50157204, BDBM50114613 (Fuji *et al* 2004); BDBM50114608, BDBM50114622, BDBM50114628, BDBM50114615, BDBM50114653, BDBM50114644, BDBM50114602 (Du *et al* 2002); BDBM50393873 (Filho *et al* 2012); BDBM50303409, BDBM35503 (Mott *et al* 2010); BDBM50007630 (Ferreira *et al* 2014).

3.2 ADME-Tox Filtering:

All the 46 compounds passed the filtering but D-gulonic acid was identified as an empty structure whereas 4, 5-di-O-caffeoyl quinic acid; (20R)-ginsenoside Rg3 and Isochlorogenic acid were duplicates. Finally, 42 compounds were screened. Although there were several criteria in this filtering tool, the following parameters were taken into consideration for screening the compounds according to *Lipinski's rule of five* and *Drug like soft filter* of FAF*Drugs*4 server (Table 1).

3.2.1 *Molecular weight (MW)*: 20 compounds were found to have MW less than 500 Da (Fig.2a).

3.2.2 *LogP or Partition coefficient between octanol and water*: 23 compounds have LogP less than 5 (fig.2b).

3.2.3 *Hydrogen Bond Acceptor (HBA)*: 23 compounds have HBA ≤ 10 (Fig.2c).

3.2.4 *Hydrogen Bond Donor (HBD)*: 19 compounds have HBD ≤ 5. (Fig.2d).

3.2.5 *Topological Polar Surface Area (tPSA)*: 23 compounds have tPSA ≤ 180. (Fig.2e).

3.2.6 *Rotatable bonds*: 39 compounds have rotatable bonds ≤ 11 (Fig.2f)

3.2.7 *Rigid bonds*: 28 compounds have rigid bonds ≤ 30.

3.2.8 *Solubility Forecast Index*: 37 compounds exhibited good solubility.

Table 1: ADMETox filtering of the compounds

Sl. No	Names of compounds	MW	LogP	HB Donors	HB Acceptors	tPSA	Rotatable bonds	Rigid bonds	Solubility Forecast index
1	1,5-di-O-caffeoyl quinic acid	516.45	1.52	7	12	214.11	9	23	Good Solubility
2	3,4-di-ocaffeoyl quinic acid	516.45	1.52	7	12	214.11	9	23	Good Solubility
3	3,5-Di-O-caffeoyl quinic acid	516.45	1.52	7	12	214.11	9	23	Good Solubility
4	(20S)-ginsenoside Rg3	785.01	4.01	9	13	218.99	10	33	Good Solubility
5	3-epimaslinic acid	472.7	6.51	3	4	80.59	1	27	Good Solubility
6	Apigenin	270.24	3.02	3	5	90.57	1	18	Good Solubility
7	Asiatic acid	488.7	5.7	4	5	100.82	2	27	Good Solubility
8	Asiaticoside	959.12	0.1	12	19	315.21	10	45	Good Solubility
9	Asiaticoside F	943.12	1.08	11	18	294.98	10	45	Good Solubility
10	Asiaticoside G	975.12	-1.3	13	20	335.44	11	45	Good Solubility
11	Bayogenin	488.7	5.84	4	5	100.82	2	27	Good Solubility
12	Brahminoside	504.7	4.36	5	6	121.05	2	27	Good Solubility
13	Cadiyenol	420.54	3.55	2	6	85.22	16	5	Good Solubility
14	Campesterol	400.68	8.8	1	1	20.23	5	20	**Reduced Solubility**

(Contd.)

(Contd.)

15	Castilliferol	432.38	4.36	4	8	137.1	5	26	**Reduced Solubility**
16	Castillicetin	464.38	3.65	6	10	177.56	5	26	**Reduced Solubility**
17	Centellasaponins B	828.98	0.41	11	16	276.52	8	39	Good Solubility
18	Centellasaponins C	959.12	-0.57	12	19	315.21	9	45	Good Solubility
19	Centellasaponins D	959.12	-0.27	12	19	315.21	10	45	Good Solubility
20	Chlorogenic acid	354.31	-0.42	6	9	167.58	5	15	Good Solubility
21	Corosolic acid	472.7	6.37	3	4	80.59	1	27	Good Solubility
22	Docosyl ferulate	502.77	12.84	1	4	55.76	25	8	**Reduced Solubility**
23	Ginsenoside Mc	754.99	4.08	8	12	198.76	10	32	Good Solubility
24	Ginsenosides Rk1	767	5.43	8	12	198.76	10	34	Good Solubility
25	Ginsenoside Rg5	767	5.37	8	12	198.76	9	34	Good Solubility
26	Ginsenoside Y	754.99	3.53	8	12	198.76	9	33	Good Solubility
27	Irbic acid	602.5	1.79	7	15	260.31	13	25	Good Solubility
28	Kaempferol	286.24	1.9	4	6	110.8	1	18	Good Solubility
29	Kaempferol-3-O-ß-D-glucoside	448.38	0.72	7	11	189.95	4	24	Good Solubility
30	Madasiatic acid	488.7	5.02	4	5	100.82	1	27	Good Solubility
31	Madecassic acid	504.7	4.36	5	6	121.05	2	27	Good Solubility
32	Madecassoside	975.12	-1.24	13	20	335.44	10	45	Good Solubility
33	Myricetin	318.24	1.18	6	8	151.26	1	18	Good Solubility
34	Pomolic acid	472.7	5.93	3	4	80.59	1	27	Good Solubility

(Contd.)

(Contd.)

35	Quadranoside IV	650.84	3.9	7	10	177.14	5	33	Good Solubility
36	Quercetin	302.24	1.54	5	7	131.03	1	18	Good Solubility
37	Rutin	610.52	-1.29	10	16	269.1	6	30	Good Solubility
38	Rosmarinic acid	360.31	2.36	5	8	147.35	7	15	Good Solubility
39	Sceffoleoside A	959.12	0.25	12	19	315.21	10	45	Good Solubility
40	Stigmasterol	412.69	8.56	1	1	20.23	5	21	**Reduced Solubility**
41	Ursolic acid	456.7	7.34	2	3	60.36	1	27	Good Solubility
42	Vitamin C	176.12	-1.85	4	6	106.89	2	6	Good Solubility

Where, **MW**= Molecular weight; **LogP**= Partition coefficient between octanol and water; **HB**= hydrogen bond; **tPSA**= Topological Polar Surface Area

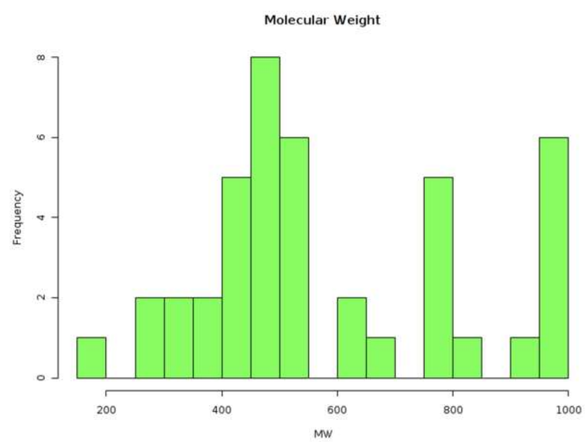


Fig. 2a

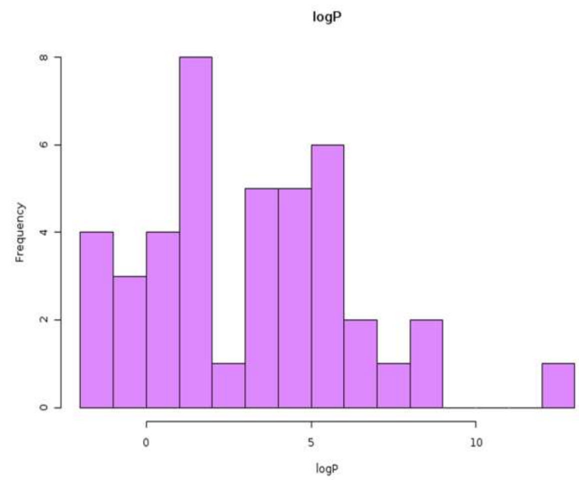


Fig. 2b

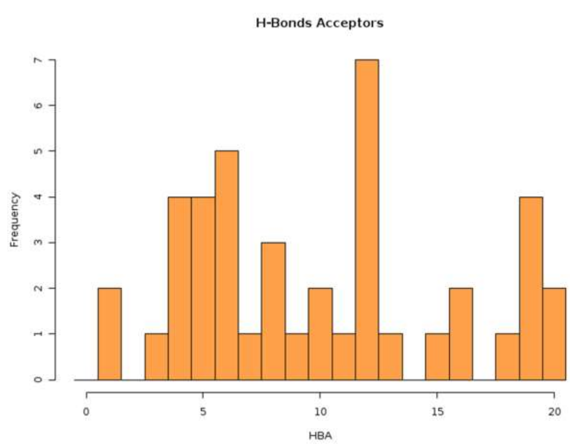


Fig. 2c

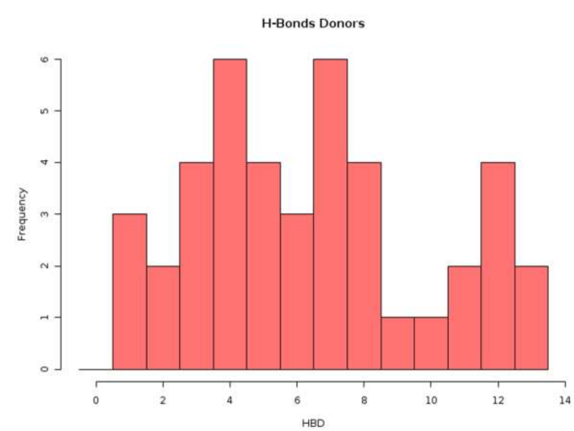
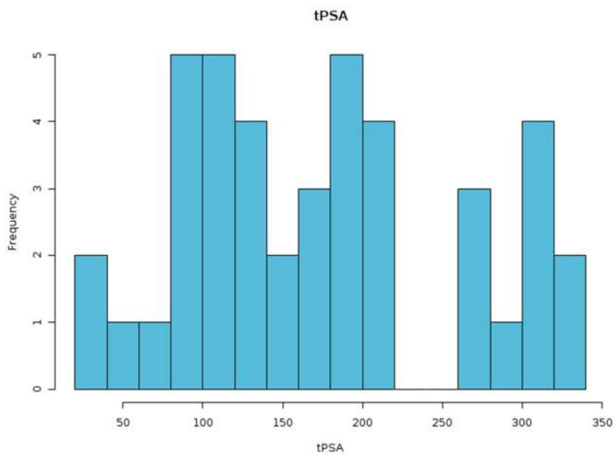


Fig. 2d



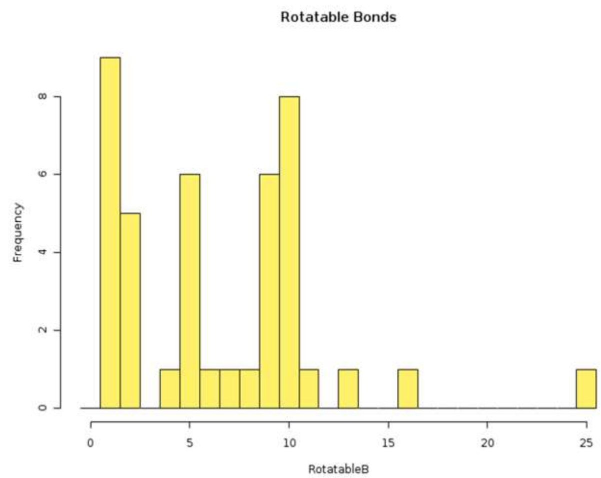


Fig. 2e **Fig. 2f**

Figure 2 (a-f) ADMETox screening. 2a: Molecular weight (daltons) of the compounds as deduced by ADMETox screening on Mobyle RPBS server. Frequency indicates the number of corresponding compounds; **2b:** the LogP of the compounds as deduced by the ADMETox Screening. As evident from the graph, most of the compounds have LogP less than 5 and hence obeys Lpinski's rule; **2c:** Illustrates the number of Hydrogen Bond Acceptors of the compounds. 23 of the compounds have HBA less than or equal to 10; **2d:** Illustrates the number of Hydrogen Bond Donors of the compounds. 19 of the compounds have HBA less than or equal to 5; **2e:** Illustrates the value of tPSA for the compounds. 23 compounds have tPSA less than or equal to 180; **2f:** Illustrates the number of rotatable bonds in the compounds. 39 compounds have rotatable bonds less than or equal to 11.

3.3 Molecular Docking:

All the compounds were used for docking with the target protein. Similarly, the inhibitors were also docked to the target. 21 compounds were able to bind to the target which are 1,5-di-O-caffeoyl quinic acid; 3,4-di-O-caffeoyl quinic acid; 3,5-Di-O-caffeoyl quinic acid; 4,5-di-O-caffeoyl quinic acid; Apigenin; Cadiyenol;

Campesterol; Castillicetin; Chlorogenic acid; D-gulonic acid; Docosyl ferulate; Ginsenoside Mc; Isochlorogenic acid; Kaempferol; Kaempferol-3-O-ß-D-glucoside; Myricetin; Quercetin; Rutin; Rosmarinic acid; Stigmasterol and Vitamin C. Strongly docked compounds were those having more negative scores. The docking scores of the 21 compounds along with bonded residue, bond energy and bond length are provided in Table.2a. Similarly, out of 25 inhibitors, 20 were able to bind to the target. The docking score of each of the inhibitors are depicted in Table.2b.

Quercetin displayed maximum binding to the target protein with a score of -11.852 (Fig.3a, b, c), followed by Apigenin having a score of -9.34 (Fig.4a, b, c). One of the inhibitors whose Binding DB ID is BDBM50303409 displayed greater binding to the target as shown by the score -12.00 (Fig.5).

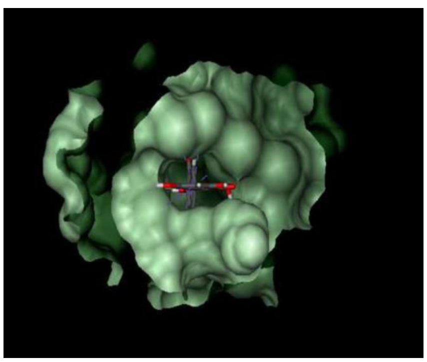


Fig.3a

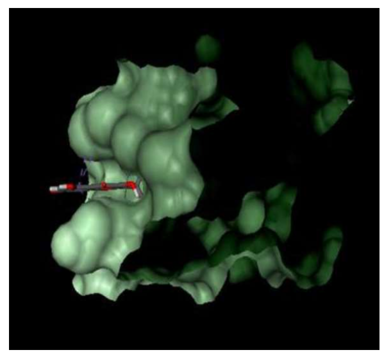


Fig.3b

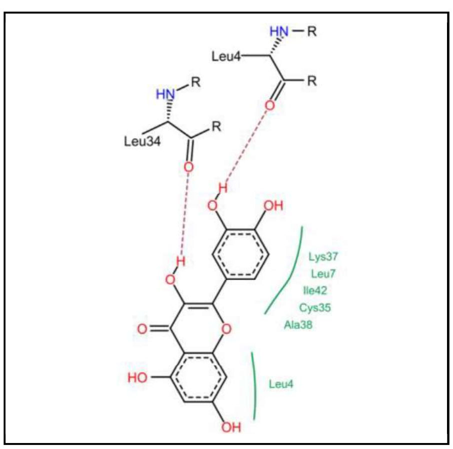


Fig.3c

Fig.3a & 3b: The 3D docking pattern of **Quercetin** with Amoebapore A**; 3c:** The pose view of docking of quercetin with the target

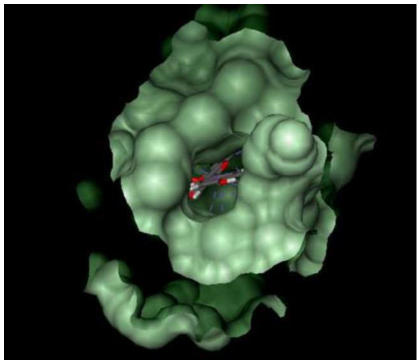


Fig.4a

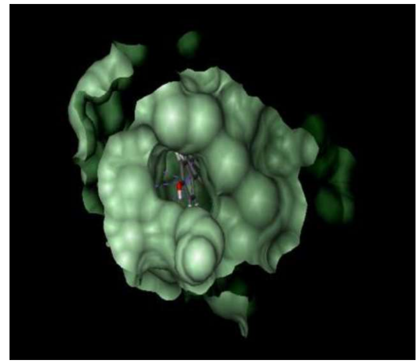


Fig.4b

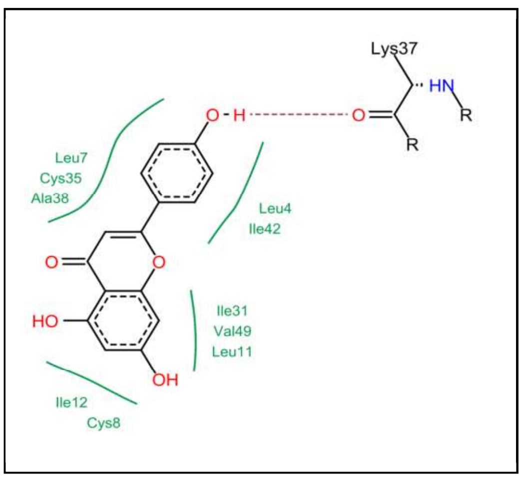


Fig.4c

Fig.4a & 4b: The 3D docking pattern of Apigenin with Amoebapore A**; 4c:** The pose view of docking of apigenin with the target

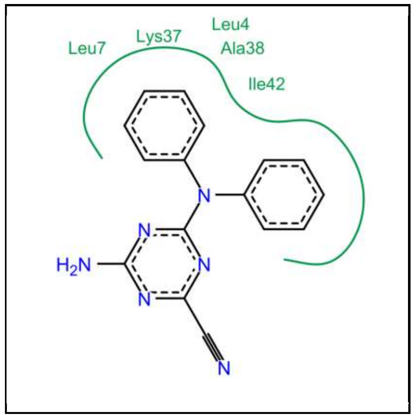


Fig.5: The pose view of docking of the strongest inhibitor, BDBM50303409 with the target

Table 2a: Docking score, bonded residues, bond length and bond energy of compounds

Phytochemical Name	DS	BR	BL	BE
1,5-di-O-caffeoyl quinic acid	2.317	H51_O LEU-4-A	1.90	-4.7
3,4-di-O-caffeoyl quinic acid	0.358	H59_O LEU-4-A	1.95	-4.7
3,5-Di-O-caffeoyl quinic acid	-3.467	H52_O LEU-4-A	1.90	-4.7
		H59_O LEU-7-A	2.13	-2.6
4,5-di-O-caffeoyl quinic acid	0.358	H59_O LEU-4-A	1.95	-4.7
Apigenin	-9.34	H30_O LYS-37-A	1.90	-3.6
Cadiyenol	15.034	O28_H ALA-38-A	1.90	-3.6
Campesterol	2.239	H71_O ALA-38-A	1.94	-2.7
Castillicetin	-1.451	H48_O LEU-4-A	1.98	-4.7
Chlorogenic acid	-5.142	H40_O ALA-38-A	2.25	-1.7
		H41_O LEU-4-A	1.92	-4.7
		H42_O LEU-4-A	1.90	-4.3
D-gulonic acid	-0.042	H20_O LEU-34-A	1.80	-4.7
		H21_O CYS-35-A	1.60	-2.6
		O10_H LYS-37-A	2.11	-3.6
		H24_O LEU-4-A	2.39	-2.4
Docosyl ferulate	10.345	H87_O LEU-45-A	1.99	-4.7
Ginsenoside Mc	20.85	H91_O ALA-38-A	1.90	-2.7
Isochlorogenic acid	-0.05	H59_O LEU-4-A	1.90	-4.7
Kaempferol	-8.361	H31_O LYS-37-A	1.90	-3.6
Kaempferol-3-O-ß-D-glucoside	5.525	H50_O LYS-37-A	1.90	-3.7
		H51_O LYS-37-A	2.17	-4.0
Myricetin	-9.016	H26_O CYS-35-A	1.75	-3.8

(Contd.)

(Contd.)

		H28_O LEU-4-A	2.24	-4.2
Quercetin	-11.852	H30_O LEU-34-A	2.26	-3.9
		H31_O LEU-4-A	2.03	-4.7
Rutin	2.001	H67_O CYS-35-A	2.35	-1.5
		H68_O LYS-37-A	1.49	-2.7
		H69_O LYS-37-A	1.63	-3.7
		H71_O ALA-38-A	2.26	-1.4
Rosmarinic acid	-5.663	H39_O LEU-4-A	1.90	-4.7
		H40_O LEU-34-A	2.24	-4.2
Stigmasterol	3.202	H65_O LYS-37-A	1.62	-4.0
Vitamin C	-5.764	H17_O LEU-4-A	2.07	-4.7
		H18_O LEU-4-A	1.77	-4.7
		H19_O LYS-37-A	2.01	-1.5
		H20_O LYS-37-A	1.66	-4.0

DS- Docking score; BR- Bonded residue; BL- Bond length (in Angstrom); BE- Bond energy. Docking scores in terms of kcal/mol.

Table 2b: Docking scores of inhibitors (controls)

ID	SCORE
BDBM50331776	1.664
BDBM19518	-6.92
BDBM50229129	-0.32
BDBM50331779	0.140
BDBM50331775	-3.57
BDBM50157204	-9.19
BDBM50114608	-10.25
BDBM50393873	-9.99
BDBM50114653	-11.55
BDBM50114644	-9.62
BDBM50303409	-12.00
BDBM50114602	-9.47

(Contd.)

BDBM35503	-3.71
BDBM50331783	1.21
BDBM50331782	-2.12
BDBM50007630	-11.77
BDBM50114622	-10.34
BDBM50114628	-9.98
BDBM50114615	-10.61
BDBM50114613	-10.34

3.4 Drug-Likeness and molecular property prediction:

Quercetin and Apigenin have been found to have drug likeliness model score of 0.93 (Fig. 6) and 0.77 (Fig. 7) respectively. MW for quercetin and apigenin are 302.04 and 270.05 whereas the logP for quercetin is 2.11 and 3.06 for apigenin. Quercetin contains 7 hydrogen bond acceptors and 5 hydrogen bond donor. On the other hand apigenin contains 5 hydrogen bond acceptor and 3 hydrogen bond donor. The overall properties are depicted in the figures as mentioned above.

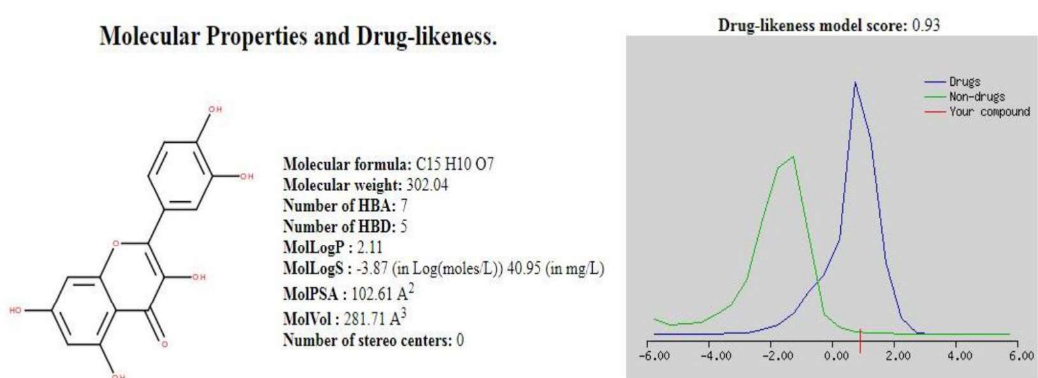


Fig.6: Drug likeliness of Quercetin (as per Molsoft L.L.C). The drug likeliness score of quercetin is 0.93.

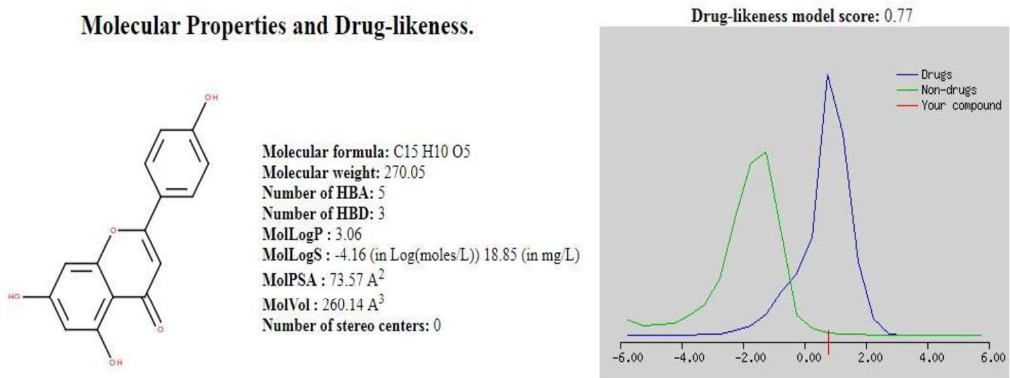


Fig.7: The drug likeliness model of Apigenin. Drug likeliness score for apigenin is 0.77

1. Discussion:

The present study evaluates the anti-amoebic potential of the compounds present in *Centella asiatica*. As already mentioned, CA has been used since ancient times as a medicinal herb to combat various diseases. For this study, the compounds have been selected through literature survey and analyzed for their binding affinity to the target protein i.e. amoebapore A, the pore forming protein of *E. histolytica*. The ADME-Tox properties of all the reported compounds have been evaluated. Lastly, the drug likeliness for the compounds have been assessed for those which showed better binding affinity to the target protein.

The phytochemicals that were selected for this study have been reported to be active in various biological processes. The inhibitors i.e. controls included in this study are well established anti protozoal agents. As evident from Fig (2a-2f), most of the compounds abide by the Lipinski's rule. A molecule to act as a drug, must have MW less than or equal to 500 daltons; HBA, HBD within 10 and 5 respectively and Log P i.e. Partition coefficient between octanol and water within 5 (Lipinski *et al* 2001; Loftsson 2015). Most of the compound screened in this study abides by the range as mentioned above. According to the *Drug-like soft* filter (Lipinski 1997; Oprea 2000, 2001; Irwin and Shoichet 2005) generated by FAF*Drugs*4, Topological Polar Surface Area or tPSA for drug like molecule lies within 180, rotatory bonds and rigid bonds lie within 11 and 30 respectively. 28 compounds belonging to CA stand by this range. In this study, most of the compounds were found to abide by this range too. As of Solubility forecast index almost all of the compounds exhibited good solubility. Thus, most of the compounds of CA display better ADMETox properties.

Molecular docking scores (kcal/mol) revealed considerable binding affinity of some compounds to the target protein as compared to the control. Thus Quercetin, followed by Apigenin exhibited stronger binding amongst the 21 docked compounds as evident from the docking score (Kitchen *et al* 2004); although one of the control seemed to have slightly stronger binding affinity towards the target. Out of 21 compounds, 12 compounds have been observed to bind with one or more Leucine (Leu) residues on the target protein (Table 2a). In case of quercetin, it has docked with the target at two leucine residues (Fig 3c) suggesting that these leucine rich region may act as active sites of amoebapore protein for binding of drug like molecules. The drug likeliness prediction of quercetin and apigenin inferred drug like properties for both since MW, logP, HBA and HBD values for these two compounds follow Lipinski's rule.
Thus, it is vivid that most of the compounds from CA have the ability to act as drug like molecules which is also evident from previous records. Amongst all the compounds from CA, Quercetin and apigenin have exhibited anti-amoebic potential since these could bind strongly to the target. Apart from this, the two compounds exhibited almost all properties that define drug like nature of a molecule.

2. Conclusion:

From this *in-silico* study it is hereby concluded that some of the compounds from CA possess anti-amoebic properties. Also, the drug like properties of CA that has been known since many years can be established by this work. CA is available easily and hence can be used as a household remedy for many diseases including amoebic dysentery and other parasitic infections but the quantity should be controlled to avoid over dosing. Further *in vivo* and *in-silico* research is necessary to establish the amoebapore inhibitory effects of CA. Herbal products are comparatively safe with respect to healing properties and ecological benefits. Keeping in mind the metabolism and bioaccumulation of synthetic drugs, it is suggested that the herbal compounds may be preferred over synthetic products. *Centella asiatica* is having multipurpose disease combatting potential and it can be further analyzed for pharmacological applications.

Acknowledgments: This work could not have completed without the guidance and suggestions of Dr. Anupam Das Talukdar, (Deputy Coordinator Bioinformatics Centre and Assistant Professor, Department of Life Science and Bioinformatics, Assam University, Silchar, Assam). The authors also acknowledge e-journals (DeLCON) access facility provided by Bioinformatics Centre, Assam University, funded by DBT, Govt. of India.

References:

Andrä J, Berninghausen O, Leippe M (2004). Membrane lipid composition protects *Entamoeba histolytica* from self-destruction by its pore-forming toxins. FEBS Lett, 564(1-2):109-15.

Babu TD, Kuttan G, Padikkala J (1995). Cytotoxic and anti-tumour properties of certain taxa of Umbelliferae with special reference to *Centella asiatica* (L.) Urban. Journal of Ethnopharmacology, 48:53-57.

Beaulieu C, Isabel E, Fortier A, Massé F, Mellon C, Méthot N, Ndao M, Nicoll-Griffith D, Lee D, Park H and Black WC (2010). Identification of potent and reversible cruzipain inhibitors for the treatment of Chagas disease. Bioorg Med Chem Lett, 20:7444-9.

Bhandari P, Kumar N, Gupta AP, Singh B, Kaul VK (2007). A rapid RP-HPTLC densitometry method for simultaneous determination of major flavonoids in important medicinal plants. J Sep Sci, 30(13):2092-2096.

Bracha R, Nuchamowitz Y and Mirelman D (2002). Amoebapore is an important virulence factor of *Entamoeba histolytica*. J. Biosci, (Suppl. 3) 27 579–587.

Chen YT, Lira R, Hansell E, McKerrow JH and Roush WR (2008). Synthesis of macrocyclic trypanosomal cysteine protease inhibitors. Bioorg Med Chem Lett, 18:5860-5863.

Chowdhury A, Sen S, Dey P, Chetia P, Talukdar A D, Bhattacharjee A and Choudhury M D (2012). Computational validation of 3-ammonio-3-(4-oxido- 1H-imidazol-1-ium-5-yl) propane-1, 1-bis (olate) as a potent anti-tubercular drug against mt-MetAP. Bioinformation, 8(18): 875-880.

Davis M J, Templeton S F, Dickensheets D L and Gross A S (2017). Massive perianal ulceration: Entamoeba histolytica and *Candida albicans* co-infection. JAAD Case Reports, 3:553-555.

Devi Prasad A G, Shyma1 T B and Raghavendra M P (2013). Plants used by the tribes for the treatment of digestive system disorders in Wayanad district, Kerala. Journal of Applied Pharmaceutical Science, 3 (08):171-175.

Du X, Guo C, Hansell E, Doyle PS, Caffrey CR, Holler TP, McKerrow JH and Cohen FE (2002). Synthesis and structure-activity relationship study of potent trypanocidal thio semicarbazone inhibitors of the trypanosomal cysteine protease cruzain. J Med Chem, 45:2695-2707.

Ekor M (2013).The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol, 4: 177. doi: 10.3389/fphar.2013.00177

Ferreira RS, Dessoy MA, Pauli I, Souza ML, Krogh R, Sales AI, Oliva G, Dias LC and Andricopulo AD (2014). Synthesis, biological evaluation, and structure-activity relationships of potent noncovalent and nonpeptidic cruzain inhibitors as anti-Trypanosoma cruzi agents. J Med Chem, 57:2380-2392.

Filho JM, Moreira DR, de Simone CA, Ferreira RS, McKerrow JH, Meira CS, Guimarães ET and Soares (2012). MB Optimization of anti-Trypanosoma cruzi oxadiazoles leads to identification of compounds with efficacy in infected mice. Bioorg Med Chem, 20:6423-6433.

Fujii N, Mallari JP, Hansell EJ, Mackey Z, Doyle P, Zhou YM, Gut J, Rosenthal PJ, McKerrow JH and Guy RK (2004). Discovery of potent thiosemicarbazone inhibitors of rhodesain and cruzain. Bioorg Med Chem Lett, 15:121-123.

Gohil K J, Patel J A, and Gajjar A K (2010). Pharmacological Review on *Centella asiatica*: A Potential Herbal Cure-all. Indian J Pharm Sci, 72(5): 546–556.

Govindan G, Sambandan TG, Govindan M, Sinskey A, Vanessendelft J, Adenan I, Rha CK (2007). A bioactive polyacetylene compound isolated from Centella asiatica. Planta Med, 73(6):597-9.

Hecht O, Nico Nuland A.V, Schleinkofer K, Dingley A J, Bruhn H, Leippe M and Grötzinger J (2004). Solution Structure of the Pore-forming Protein of *Entamoeba histolytica.* The Journal of Biological Chemistry 279:17834-17841.

Irwin JJ and Shoichet BK (2005). ZINC - A Free Database of Commercially Available Compounds for Virtual Screening, J Chem Inf Model, 45 (1):177-182.

Jagtap NS, Khadabadi SS, Ghorpade DS, Banarase NB, Naphade SS (2009). Antimicrobial and Antifungal Activity of *Centella asiatica* (L.) Urban, Umbeliferae. Research J Pharm Tech, 2 (2): 328-330.

James JT and Dubery IA (2009). Pentacyclic triterpenoids from the medicinal herb, *Centella asiatica* (L.) Urban. Molecules, 14(10):3922-41.

Kadir MF, Bin Sayeed MS, Setu NI, Mostafa A, Mia MM (2014). Ethnopharmacological survey of medicinal plants used by traditional health practitioners in Thanchi, Bandarban Hill Tracts, Bangladesh. J Ethnopharmacol, 155(1):495-508.

Kitchen D B, Decornez H, Furr J R. and Bajorath J (2004). Docking and Scoring in Virtual Screening for Drug Discovery: Methods and Applications. Nat Rev Drug Discov, 3(11):935-949.

Leippe M (1997). Amoebapores. Parasitology Today, 13(5), 178-183.

Lipinski CA, Lombardo F, Dominy BW and Feeney PJ (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev, 46(1-3):3-26.

Loftsson T (2015). Physicochemical Properties and Pharmacokinetics. In: Thorsteinn Loftsson, ed. Essential Pharmacokinetics, Elsevier Inc., USA, pp. 85-104.

Lynch EC, Rosenberg IM and Gitler C (1982). An ion-channel forming protein produced by *Entamoeba histolytica*. The EMBO Journal, 1(7): 801-804.

Matsuda H, Morikawa T, Ueda H, Yoshikawa M (2001). Medicinal foodstuffs. XXVII. Saponin constituents of gotu kola (2): structures of new ursane- and oleanane-type triterpene oligoglycosides, centellasaponins B, C, and D, from *Centella asiatica* cultivated in Sri Lanka. Chem Pharm Bull (Tokyo), 49(10):1368-1371.

Mott BT, Ferreira RS, Simeonov A, Jadhav A, Ang KK, Leister W, Shen M, Silveira JT, Doyle PS, Arkin MR, McKerrow JH, Inglese J, Austin CP, Thomas CJ, Shoichet BK and Maloney DJ (2010). Identification and optimization of inhibitors of Trypanosomal cysteine proteases: cruzain, rhodesain, and TbCatB. J Med Chem, 53:52-60.

Nhiem NX, Tai BH, Quang TH, Kiem PV, Minh CV, Nam NH, Kim JH, Im LR, Lee YM, Kim YH (2011). A new ursane-type triterpenoid glycoside from Centella asiatica leaves modulates the production of nitric oxide and secretion of TNF-α in activated RAW 264.7 cells. Bioorg Med Chem Lett, 21(6):1777-81.

Oprea TI (2000). Property distribution of drug-related chemical databases. J Comput Aided Mol Des, 14(3):251-264.

Oprea TI (2001). Is There a Difference between Leads and Drugs? A Historical Perspective. J Chem Inf Comput Sci, 41 (5):1308-1315.

Orhan I E (2012). *Centella asiatica* (L.) Urban: From Traditional Medicine to Modern Medicine with Neuroprotective Potential. Evidence-Based Complementary and Alternative Medicine. http://dx.doi.org/10.1155/2012/946259

Pittella F, Dutra RC, Junior DD, Miriam, Lopes TP and Barbosa NR (2009). Antioxidant and cytotoxic activities of *Centella asiatica* (L), Int J Mol Sci, 10:3713-3721.

Rafamantanana MH, Rozet E, Raoelison GE, Cheuk K, Ratsimamanga SU, Hubert P, Quetin-Leclercq J (2009). An improved HPLC-UV method for the simultaneous quantification of triterpenic glycosides and aglycones in leaves of *Centella asiatica* (L.) Urb (APIACEAE). J Chromatogr B Analyt Technol Biomed Life Sci, 877(23):2396-402.

Ralston KS and Petri WA Jr. (2011). Tissue destruction and invasion by *Entamoeba histolytica*. Trends Parasitol, 27(6): 254–263.

Satake T, Kamiya K, An Y, Oishi Nee Taka T, Yamamoto J (2007). The anti-thrombotic active constituents from *Centella asiatica*. Biol Pharm Bull, 30(5):935-40.

Singh S, Singh DR, Salim KM, Srivastava A, Singh LB, Srivastava RC (2011). Estimation of proximate composition, micronutrients and phytochemical compounds in traditional vegetables from Andaman and Nicobar Islands. Int J Food Sci Nutr, 62(7):765-73.

Sushen U, Chouhan A, Ali K and Ranjesh V (2017). Medicinal properties of *centella asiatica* (l.): a review. Ejpmr, 4(9):261-268.

Wanasuntronwong A, Tantisira MH, Tantisira B, Watanabe H (2012). Anxiolytic effects of standardized extract of *Centella asiatica* (ECa 233) after chronic immobilization stress in mice. J Ethnopharmacol, 143(2):579-585.

Weng XX, Shao Y, Chen YY, Gao W, Cheng L, Kong DY (2011). Two new dammarane monodesmosides from *Centella asiatica*. J Asian Nat Prod Res, 13(8):749-755.

William AP Jr. (2008). Intestinal invasion by *Entamoeba histolytica* Shahram Solaymani Mohammadi. Subcell Biochem, 47: 221–232.

Yoshida M, Fuchigami M, Nagao T, Okabe H, Matsunaga K, Takata J, Karube Y, Tsuchihashi R, Kinjo J, Mihashi K and Fujioka T (2005). Antiproliferative constituents from Umbelliferae plants VII. Active triterpenes and rosmarinic acid from *Centella asiatica*. Biol Pharm Bull, 28(1):173-175.

Yu QL, Gao WY, Zhang YW Teng J and Duan HQ (2007). Studies on chemical constituents in herb of *Centella asiatica.* Zhongguo Zhong Yao Za Zhi, 32(12):1182-4.

How to cite this article:

Choudhury B P, Laskar M A, Choudhury M D. Pharmacological screening of *Centella asiatica* for its anti-amoebic properties: An in-silico approach. *Curr Trends Pharm Res,* 2018, 5(1):1-23.