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QSAR modeling for prediction of acute toxicity and mutagenicity in different test models by established common phytochemicals present in *Phyllanthus niruri*

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ABSTRACT

In globe, *Pyllanthus niruri* is a well-established medicinal herb studied by many researchers, grown widely in many parts of West Bengal. The present study was aimed to predict the acute toxicity as LC_{50} in *Daphnia magna* and *Pimephales promelas* and rat oral LD_{50} value as well as Ames mutagenicity by using QSAR modeling software, T.E.S.T. (Toxicity Estimation Software Tool) for commonly found phytochemicals in *Pyllanthus niruri*. In present works, the data were obtained for LC_{50}, few phytochemicals were toxic to *D. magna* and *P. promelas* and also mutagenic but rat oral LD_{50} determined less toxic. The present QSAR modeling work is suggesting that more researches should be required through experimental as well as predictive study with other prescribed software to know the mechanisms of toxicity and mutagenicity for these combined form of phytochemicals after separating each natural chemical from extract prior to drugs development for therapeutic usage.

Keywords: QSAR modeling; Common phytochemicals; *Phyllanthus niruri*; T.E.S.T. software; Predictive toxicity and mutagenicity

1. INTRODUCTION

Plants have been widely used in traditional medicine since a long time for anti-carcinogenicity and anti-mutagenicity (Agarwal and Pandey, 2009; Das et al., 2014). It has already been reported in globe major plant species used as drugs for their phytochemicals and several herbal drugs are derived from plants in the form of hot liquids as an extract or pure substances (Adjanohoum, 1982; Oksman-Caldentey and Barz, 2002).

The plant-based chemicals represent a source for herbal medicines and many investigations are still in process with these extracts for several therapeutic potential. These phytochemicals are suitable in the treatment of bacterial and/or fungal diseases while synthetic chemicals have several side effects to animals (Iwu *et al*, 1999). According to the researchers, new drugs contain as a lead compound of phytochemicals for the pharmaceutical industry (Aiyelaagbe, 2001; Aiyoro et al, 2008).

In West Bengal, one of the most commonly found medicinal plant *Phyllanthus niruri* Linn belongs to family Euphorbiaceae, known as 'stonebreaker' due to its antilithic property. *P. niruri* is a small erect annual herb growing up to 30–40 cm in maximum height. This plant species is indigenous to the rainforest of Amazon and other tropical areas, mainly South East Asia, Southern part of India and China (Girach et al., 1994) and people used as medicines by their traditional knowledge over 2000 years before.

The extracts of whole plant body is used as therapeutic purposes for many diseases viz. gonorrhoea, frequent menstruation, diabetes, severe diarrhea, influenza, vaginal inflammation, tumours, diuretics, jaundice, kidney stones or nephrolith and dyspepsia (Dhar et al., 1968; Bagalkotkar et al., 2006).

According to Chopra et al. (1986), liver toxicity, hepatitis B, high sugar level, viral and bacterial diseases can be prevented by using extracts of *P. niruri*. In India, this species is called as Pitirishi or Budhatri or Bhui amla, is used commonly for household therapy of several diseases viz. lung diseases, extreme thirst, haemoglobin deficiency, jaundice etc. (Dhar et al., 1968).

It has already been established that phytochemicals mainly polyphenols, alkaloids, flavonoids, terpenoids, lignans, tannins, coumarins and saponins are found from various parts of *P. niruri* in a combined form that have a potential of protection against diseases (Bagalkotkar et al., 2006). All of these phytochemicals are natural in origin, may be antimutagenic and/or anticarcinogenic but sometimes few of them may show toxic and mutagenic positive to biota.

Acute toxicities and mutagenesis are major toxicity endpoints that supported in ecotoxicological research era. Moreover, toxicity and mutagenicity endpoints can be studied and predicted through QSAR (Quantitative Structure Activity Relationships) modeling software (Talapatra et al., 2015; Talapatra and Sarkar, 2015; Banerjee and Talapatra, 2015), T.E.S.T. (Toxicity Estimation Software Tool) is one of the 2D molecular descriptor based software (USEPA, 2012).

The present study was attempted to predict the acute toxicity as LC_{50} in *Daphnia magna* and *Pimephales promelas* and rat oral LD_{50} value as well as mutagenicity positive or negative in *Salmonella typhimurium* of common established phytochemicals present in *Pyllanthus niruri*. The prediction was carried out by using QSAR modeling software, T.E.S.T.

2. MATERIALS AND METHODS

In the present study, the selection of phytochemicals in *P. niruri* were done on the basis of literature study (Bagalkotkar et al., 2006). In Table 1, all 30 types of phytochemicals were listed alongwith CAS no., SMILES and molecular structures were tabulated, which obtained from ChemIDPlus database. The established common 30 types of phytochemicals were selected. The software T.E.S.T, Version 4.1 was used in the present study (USEPA, 2012). The present study was carried out to predict acute toxicity as the LC_{50} values in cladocera, *Daphnia magna* and fathead minnow (cyprinid fish), *Pimephales promelas* and LD_{50} value of oral exposure in rat as well as the prediction of mutagenicity positive or negative on *Salmonella typhimurium* of these phytochemicals (Table 2).

The values for acute toxicity and mutagenicity study was tabulated after obtaining predictive data of individual compound from T.E.S.T. software. All the data were obtained by consensus method, which is basically the average predicted LC_{50}, LD_{50} and mutagenicity positive or negative values are calculated from average inbuilt QSAR methodologies, which are mainly hierarchical clustering method, the FDA MDL method and nearest neighbor methods (USEPA, 2012). In present software, the structure of studied chemical can only be seen when writing CAS registry no. of individual chemical in appropriate place followed by clicking enter key. The predicted value of consensus method can only be obtained after calculating internally the software. As per Martin et al. (2008), this software contains of 7,420 chemicals' database, have already been programmed and 2-dimensional molecular descriptors of 797 nos. (Zhu et al., 2009; USEPA, 2012). For mutagenicity estimation, a dataset of 6512 chemicals have compiled by Hansen et al. (2009) and the final dataset consisted of 5743 chemicals (without salts, mixtures, ambiguous compounds, and compounds without CAS numbers) in T.E.S.T.

3. RESULTS AND DISCUSSION

The present study was done on 30 types of common phytochemicals have already reported from *P. niruri* plant (Bagalkotkar et al., 2006). The images are depicted in Fig 1a and 1b for top view of *Phyllanthus* sp. and close view of fruits and flowers. All the compounds were selected on the basis of commonly available in extracts. These are 5 alkaloids (norsecurinine, *β*-glucogallin, phyllochrysine, anthraquinone and nirurin), 6 flavonoids (rutin, quercetin, quercitrin, astragalin, catechin and quercetol), 3 steroids (β-sitosterol, cholesterol and estradiol), 1 polyphenols (diosgenin), 3 tapenoids (limolene, lupeol and p-cymene), 5 lignans (phyllanthine, hypophyllantine, nirtetralin, nirphyllin and phyllnirurin), 3 tannins (geraniin, corilagin and triacontanol), 3 coumarins (ellagic acid, gallic acid and methyl brevifolincarboxylate) and 1 other compound (methyl salicylate). The CAS (Chemical Abstracts Services) no., SMILES and structure were tabulated in Table 1.

In Table 2, the acute toxicity (LC_{50}) prediction data in *D. magna* and *P. promelas* and rat oral LD_{50} value as well as predicted data of mutagenicity were tabulated for above mentioned phytochemicals and these compounds were selected along with their CAS no. Out of the 30 common phytochemicals, all the predicted data for LC_{50} (mg/l), LD_{50} (mg/kg) and mutagenicity were obtained by using T.E.S.T. for only 15 compounds namely anthraquinone, rutin, quercetin, quercitrin, quercetol, β-sitosterol, cholesterol, estradiol, diosgenin, limolene,

lupeol, p-cymene, ellagic acid, gallic acid and methyl salicylate. Rest 15 compounds viz. norsecurinine, *β*-glucogallin, phyllochrysine, phyllnirurin, nirurin, astragalin, catechin, phyllanthine, hypophyllantine, nirtetralin, nirphyllin, geraniin, corilagin, triacontanol and methyl brevifolincarboxylate were unable to predict because unidentified CAS no. in the present software.

Among several medicinal plants, *Phyllanthus nururi* has potent medicinal properties due to the presence of flavonoids, polyphenols, alkaloids, steroids etc. (Chopra et al., 1986; Harish and Shivanandappa, 2006). According to Kumaran and Karunakaran (2007), five species under the genus *Phyllanthus* have high antioxidant properties. The presence of polyphenols, flavonoids etc. in plants have showed antimutagenic abilities in biota (Thompson et al. 1984; Lim and Murtijaya, 2007).

The present study concerns about phytochemicals as individually how much safe for medicinal usage? If these discharge directly into waterbodies then what happens to aquatic biota like daphnids, fish etc? How bioassay test through rat oral LD_{50} detect acute toxicity prior to human trial in respect to herbal drugs development? All answers are still unknown because many chemicals in alkaloids, sterols, lignans etc. have been proved toxic, mutagenic and carcinogenic and also induce tumour (Normen et al., 2001; Valerio et al., 2010; Bode and Dong, 2015). The present research works on QSAR modeling to predict acute toxicity and mutagenicity for natural chemicals (phytochemicals) are supported by Arvidson et al. (2008). According to them, experimental toxicity data validated by computational toxicological software accurately for six types of natural products such as estragole, pulegone, aristolochic acid, lipoic acid, 1-octacosanol and epicatechin). In other words, β-sisterol and stigmsterol were reported for colon and rectal cancer in human (Normen et al., 2001), cholesterol has also induced cancer growth in vitro cell lines study (Ifere et al., 2010).

For *D. magna*, it was observed the predicted LC_{50} data (mg/l) for anthraquinone (3.41), rutin (0.92), quercetin (0.53), quercitrin (0.72), quercetol (0.53), β-sitosterol (0.07), cholesterol (0.19), estradiol (0.15), diosgenin (0.28), limolene (2.17), lupeol (0.14), p-cymene (5.86), ellagic acid (2.77), gallic acid (31.79) and methyl salicylate (27.81) while in *P. promelas*, anthraquinone (5.01), rutin (0.004), quercetin (0.53), quercitrin (0.78), quercetol (0.78), β-sitosterol (0.17), cholesterol (0.44), estradiol (0.98), diosgenin (1.20), limolene (1.34), lupeol (0.20), p-cymene (6.04), ellagic acid (0.29), gallic acid (91.34) and methyl salicylate (29.16) respectively (Table 2).

In case of rat oral LD_{50} data (mg/kg), anthraquinone (4450.09), rutin (2589.12), quercetin (2639.57), quercitrin (1763.88), quercetol (2639.57), β-sitosterol (894.28), cholesterol (1879.56), estradiol (1186.18), diosgenin (106.87), limolene (4286.15), lupeol (610.81), p-cymene (3265.39), ellagic acid (1513.19), gallic acid (3912.42) and methyl salicylate (2140.03) respectively (Table 2).

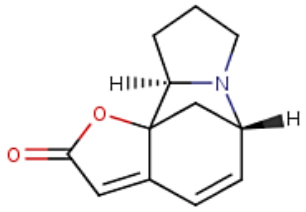
The mutagenicity prediction data were observed that out of 15 compounds 5 compounds were obtained mutagenic positive viz. anthraquinone (0.74), quercetin (0.55), quercitrin (0.54), quercetol (0.55) and ellagic acid (0.67) while 10 compounds were found mutagenic negative viz. rutin (0.06), β-sitosterol (0.25), cholesterol (0.16), estradiol (0.23), diosgenin (0.16), limolene (0.00), lupeol (0.19), p-cymene (0.06), gallic acid (0.31) and methyl salicylate (0.15) respectively (Table 2).

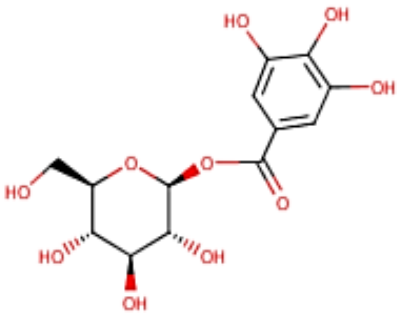
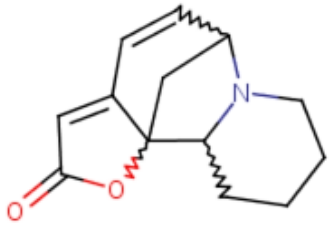
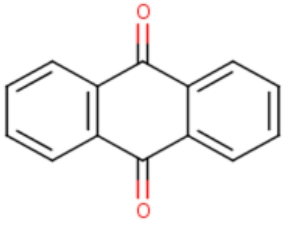
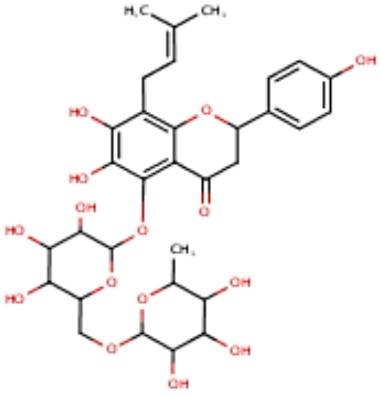
In present results for *D. magna*, predicted acute toxicity (LC_{50}) values were obtained in order methyl salicylate > gallic acid > p-cymene > anthraquinone > ellagic acid > limolene > rutin > quercitrin > quercetin and quercetol > diosgenin > cholesterol > estradiol > lupeol > β-

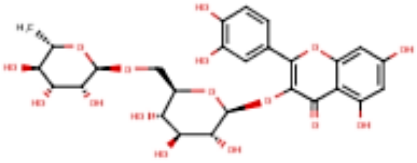
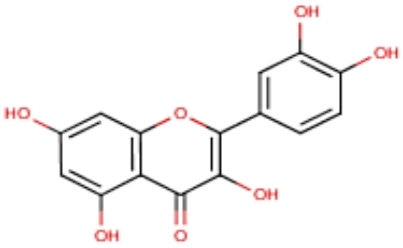
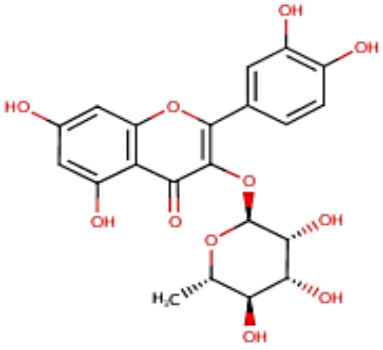
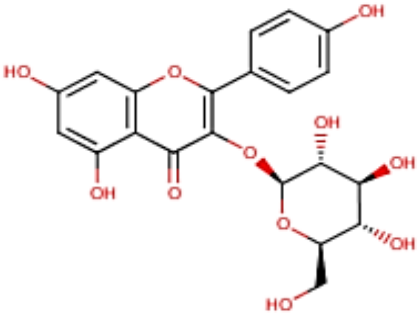
sitosterol while in *P. promelas*, gallic acid > methyl salicylate > p-cymene > anthraquinone > limolene > diosgenin > estradiol > quercetol and quercitrin > quercetin > cholesterol > lupeol > β-sitosterol > rutin (Table 2). The ecological roles of few phytochemicals such as alkaloids, sterols, polyphenol etc. have showed potent toxic effect to insects and other chordates (Kennedy and Wightman, 2011), the present results with an agreement to obtained toxicity in *D. magna* and *P. promelas*. In case of rat oral LD_{50} data, it was observed acute toxicity in the order anthraquinone > limolene > gallic acid > p-cymene > quercetol > quercetin > rutin > methyl salicylate > cholesterol > quercitrin > ellagic acid > estradiol > β-sitosterol > lupeol > diosgenin (Table 2). The predictive results were obtained not much toxic when orally exposed to rat, this data may be the activity of detoxification mechanisms, metabolic activities etc. and/or selection of 2-dimensional molecular descriptors by the T.E.S.T. for mammal like rat. According to Bode and Dong (2015), several compounds from natural origin (plant products) have already been reported potential cancer causing agents.

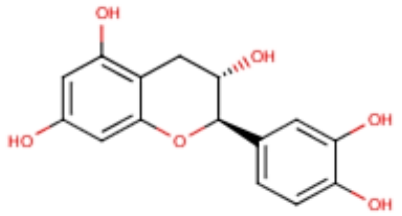
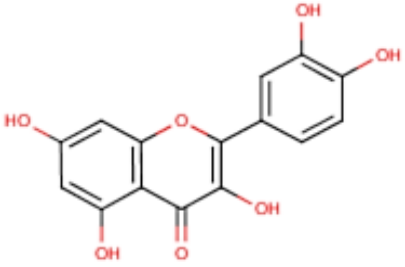
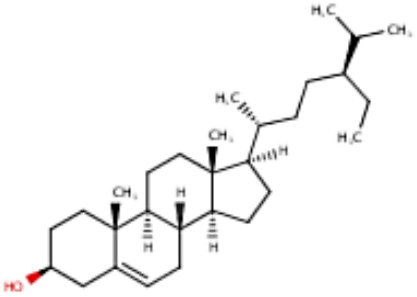
Generally combinations of phytochemicals by plant extracts act as antimutagenic in bacterial strains. These phytochemicals has developed for drugs used in cancer and/or several diseases prevention. The herb, *Phyllanthus niruri* plant is one of suitable example for phyto medicines (Syed Asad et al., 2012; Husain et al., 2014), but in present results, the mutagenicity prediction for individual phytochemical data obtained 5 compounds were mutagenic positive when studied individually. These are in order quercitrin > anthraquinone > ellagic acid > quercetin and quercetol while 10 compounds were found mutagenic negative (Table 2). The previous study of QSAR modeling supported present results that few individual phytochemical observed mutagenic itself (Banerjee and Talapatra, 2015). Moreover, it was first reported by Indian and Brazilian researchers regarding the medicinal properties of *P. niruri* and this plant species is endemic in both areas, as a historical feedback of medicinal use traditionally by local inhabitants (Unander et al., 1991). The alkaloid of *P. niruri* is having antispasmodic activity have documented by Calixto et al. (1984). In the late 1980s, *P. niruri* served as medicinally important against hepatitis B disease throughout the globe (Venkateswaran et al., 1987). The present QSAR modeling work is suggesting that more researches should be needed to know the mechanisms of toxicity and mutagenicity by few phytochemicals before drugs development for therapy.

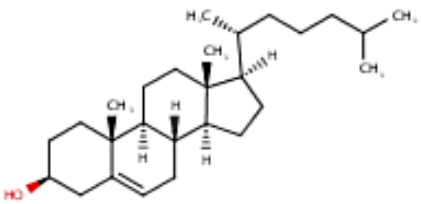
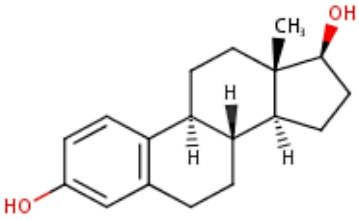
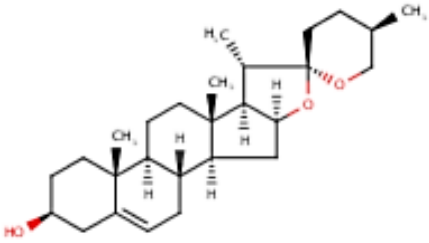
Table 1. List of phytochemicals found in *Phyllanthus niruri*.

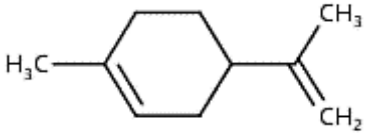
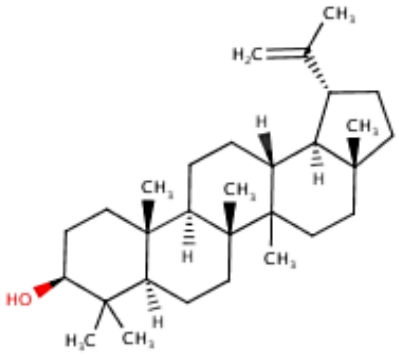
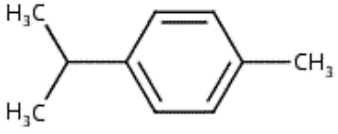
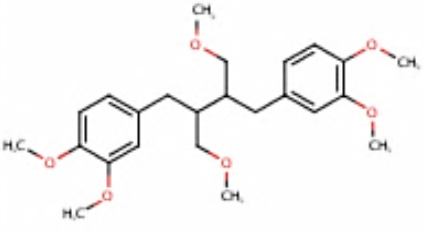
Sl. No.	Compounds	CAS No.*	SMILES*	Structure*
Alkaloids				
1.	Norsecurinine	2650-35-3	C1=2[C@@]3([C@@H]4[N@@]([C@@H](C=C1)C3)CCC4)OC(C2)=O	

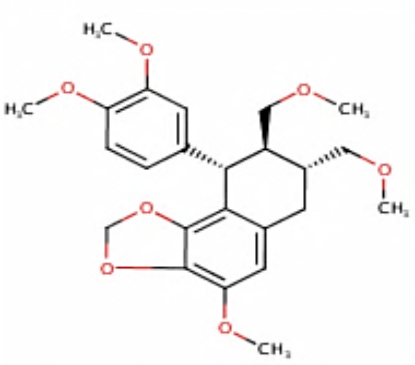
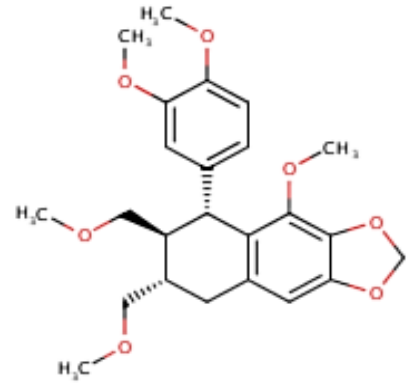
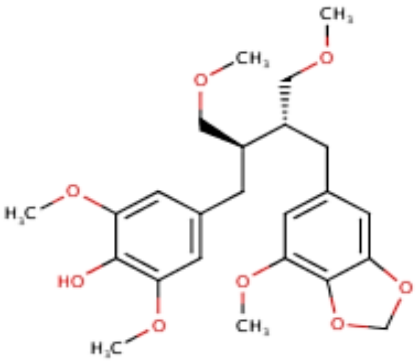
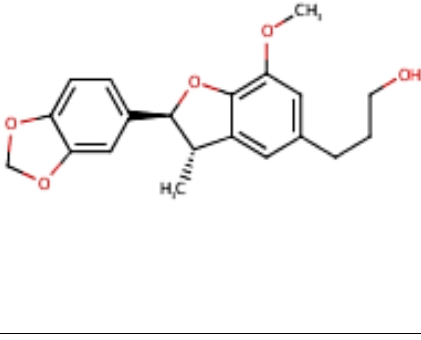
2.	*β*-Glucogallin	13405-60-2	O1[C@H]([C@@H]([C@@H](O)[C@@H]([C@H]1CO)O)O)OC(c1cc (O)c(c(c1)O)O)=O	
3.	Phyllochrysine	884-68-4	C1=CC2=CC(=O)OC32C2N(C1C3)CCCC2	
4.	Anthraquinone	84-65-1	c12c(C(c3ccccc3C1=O)=O)cccc2	
5.	Nirurin	96253-68-8	c1(c(c(O[C@@H]2[C@@H]([C@@H]([C@@H]([C@@H]O2)CO[C@@H]2[C@@H]([C@@H]([C@@H]([C@@H](O2)C)O)O)O)O)O)O)O)c2c(c1C\C=C(\C)C)O[C@@H](c1ccc(cc1)O)CC2=O)O)O	
Flavonoids				

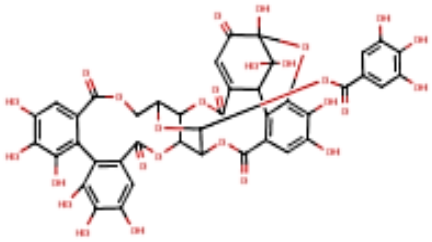
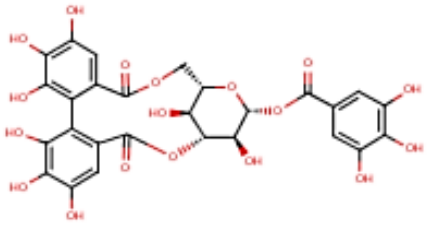
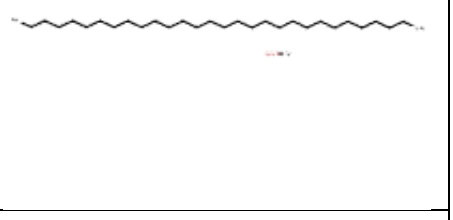
6.	Rutin	153-18-4	c1(c(c(c2c(cc(cc2o1)O)O)=O)O[C@@H]1O[C@H](CO[C@H]2[C@@H]([C@@H]([C@@H](O)[C@@H](O2)C)O)O)[C@@H](O)[C@@H]([C@H]1O)O)c1cc(c(O)cc1)O	
7.	Quercetin	117-39-5	c12c(oc(c3cc(c(O)cc3)O)c(c1=O)O)cc(O)cc2O	
8.	Quercitrin	522-12-3	c1(c(c(=O)c2c(o1)cc(cc2O)O)O[C@H]1[C@@H]([C@@H]([C@H]([C@@H](O1)C)O)O)O)c1cc(c(cc1)O)O	
9.	Astragalin	480-10-4	O[C@H]1[C@H](Oc2c(c3c(cc(cc3oc2c2ccc(cc2)O)O)O)=O)O[C@H](CO)[C@H](O)[C@@H]1O	

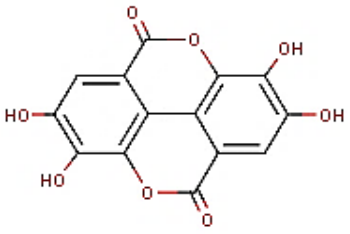
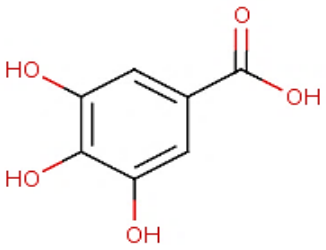
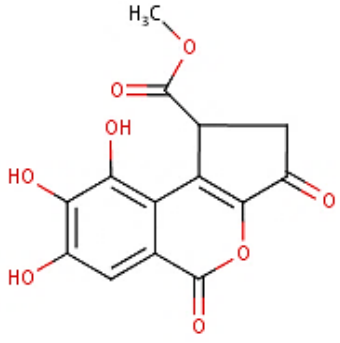
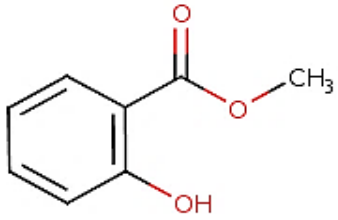
10.	Catechin	7295-85-4	c1cc(c(cc1[C@@H]2[C@H](Cc3c(cc(cc3O2)O)O)O)O)O	
11.	Quercetol	117-39-5	c12c(oc(c3cc(c(O)cc3)O)c(c1=O)O)cc(O)cc2O	
Steroids				
12.	β-Sitosterol	83-46-5	[C@@]12([C@H]([C@H]3[C@@H]([C@@]4(C(=CC3)C[C@H](CC4)O)C)CC2)CC[C@@H]1[C@@H](CC[C@@H]([C@H](C)C)CC)C)C	

13.	Cholesterol	57-88-5	C[C@H](CCCC(C)C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C	
14.	Estradiol	50-28-2	C[C@]12CC[C@@H]3c4ccc(cc4CC[C@H]3[C@@H]1CC[C@@H]2O)O	
Polyphenols				
15.	Diosgenin	512-04-9	[C@@]12([C@H]([C@H]3[C@@H](O1)C[C@@H]1[C@@]3(CC[C@H]3[C@H]1CC=C1[C@@]3(CC[C@@H](C1)O)C)C)CC[C@H](CO2)C	
Terpenoids				

16.	Limonene	138-86-3	C([C@@H]1CCC(C)=CC1)(C)=C	
17.	Lupeol	545-47-1	C1([C@@H]2CC[C@@]3([C@@]4([C@@H]([C@H]5[C@H](C(=C)C)CC[C@@]5(CC4)C)CC[C@H]3[C@@]2(CC[C@@H]1O)C)C)C)(C)C	
18.	p-cymene	99-87-6	c1(C(C)C)ccc(C)cc1	
Lignans				
19.	Phyllanthine	10351-88-9	c1(c(ccc(c1)C[C@@H]([C@@H](Cc1cc(OC)c(cc1)OC)COC)COC)OC)OC	

20.	Hypophyllantine	33676-00-5	c1(c(ccc([C@@H]2[C@H]([C@H](COC)Cc3c2c2c(OCO2)c(OC)c3)COC)c1)OC)OC	
21.	Nirtetralin	50656-78-5	O1COc2c1cc1C[C@@H]([C@H]([C@@H](c1c2OC)c1cc(c(cc1)OC)OC)COC)COC	
22.	Nirphyllin	120396-54-5	c1(c(cc(cc1OC)C[C@H]([C@@H](Cc1cc2OCOc2c(c1)OC)COC)COC)OC)O	
23.	Phyllnirurin	120396-53-4	O1[C@@H]([C@H](c2c1c(cc(c2)CCCO)OC)C)c1cc2OCOc2cc1	

Tannins				
24.	Geraniin	60976-49-0	C1(O[C@@H]2[C@@H]3COC(=O)c4cc(O)c(O)c(O)c4c4c(cc(c(c4O)O)O)C(O[C@@H]2[C@@H](OC(=O)c2c4[C@@H]5C([C@@](Oc4c(O)c(O)c2)(C(=O)C=C15)O)(O)O)[C@@H](O3)OC(=O)c1cc(c(c(c1)O)O)O)=O)=O	
25.	Corilagin	23094-69-1	c1c(cc(c(c1O)O)O)C(=O)O[C@@H]2[C@H]([C@H]3[C@H]([C@@H](O2)COC(=O)c4cc(c(c(c4-c5c(cc(c(c5O)O)O)C(=O)O3)O)O)O)O)O	
Saponins				
26.	Triacontanol	28351-05-5	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCC.*O	
Coumarins				

27.	Ellagic acid	476-66-4	c12c3c4oc(c2cc(c(c1oc(c3cc(c4O)O)=O)O)O)=O	
28.	Gallic acid	149-91-7	c1(cc(c(O)c(c1)O)O)C(O)=O	
29.	Methyl brevifolincarboxylate	154702-76-8	C1(=O)c2oc(c3cc(O)c(c(c3c2[C@@H](C(=O)OC)C1)O)O)=O	
Other compound				
30.	Methyl salicylate	119-36-8	c1(c(cccc1)O)C(OC)=O	

*All data obtained from ChemIDPlus

Table 2. Prediction of different types of toxicity and mutagenicity in recommended test species by common phytochemicals in *Phyllanthus nuriri*.

Sl No.	Phytochemicals	Predictive acute toxicity (LC_{50}) values (mg/l) in *D. magna* by T.E.S.T.	Predictive acute toxicity (LC_{50}) values (mg/l) in *P. promelas* by T.E.S.T.	Predictive acute toxicity rat oral (LD_{50}) values (mg/kg) by T.E.S.T	Predictive mutagenicity values in S. typhimurium by T.E.S.T
1.	Norsecurinine	N.A.	N.A.	N.A.	N.A.
2.	*β*-Glucogallin	N.A.	N.A.	N.A.	N.A.
3.	Phyllochrysine	N.A.	N.A.	N.A.	N.A.
4.	Anthraquinone	3.41	5.01	4450.09	0.74 (+)
5.	Phyllnirurin	N.A.	N.A.	N.A.	N.A.
6.	Nirurin	N.A.	N.A.	N.A.	N.A.
7.	Rutin	0.92	0.004	2589.12	0.06 (-)
8.	Quercetin	0.53	0.78	2639.57	0.55 (+)
9.	Quercitrin	0.72	0.16	1763.88	0.54 (+)
10.	Astragalin	N.A.	N.A.	N.A.	N.A.
11.	Catechin	N.A.	N.A.	N.A.	N.A.
12.	Quercetol	0.53	0.78	2639.57	0.55 (+)
13.	β-Sitosterol	0.07	0.17	894.28	0.25 (-)
14.	Cholesterol	0.19	0.44	1879.56	0.16 (-)
15.	Estradiol	0.15	0.98	1186.18	0.23 (-)
16.	Phyllanthine	N.A.	N.A.	N.A.	N.A.
17.	Hypophyllantine	N.A.	N.A.	N.A.	N.A.
18.	Nirtetralin	N.A.	N.A.	N.A.	N.A.
19.	Nirphyllin	---	---	---	---
20.	Diosgenin	0.28	1.20	106.87	0.16 (-)

21.	Limolene	2.17	1.34	4286.15	0.00 (-)
22.	Lupeol	0.14	0.20	610.81	0.19 (-)
23.	p-cymene	5.86	6.04	3265.39	0.06 (-)
24.	Geraniin	N.A.	N.A.	N.A.	N.A.
25.	Corilagin	N.A.	N.A.	N.A.	N.A.
26.	Triacontanol	N.A.	N.A.	N.A.	N.A.
27.	Ellagic acid	2.77	0.29	1513.19	0.67 (+)
28.	Gallic acid	31.79	91.34	3912.42	0.31 (-)
29.	Methyl brevifolincarboxylate	N.A.	N.A.	N.A.	N.A.
30.	Methyl salicylate	27.81	29.16	2140.03	0.15 (-)

N.A. = Not available in T.E.S.T. software

Fig. 1(a). The medicinal herb, *Phyllanthus* sp. (Top view).

Fig. 1(b). The medicinal herb, *Phyllanthus* sp. (Close view of fruits and flowers).

4. CONCLUSIONS

The present work concluded that *P. niruri* plant contents several phytochemicals for disease prevention (Venkateswaran et al., 1987; Syed Asad et al., 2012; Husain et al., 2014) but the predictive acute toxicity study, the LC_{50} values of few phytochemicals were obtained toxic in *D. magna* and *P. promelas* as well as 5 compounds were observed mutagenic while few were less toxic to rat oral exposure (LD_{50} values).

All the predictive toxicity and mutagenicity data were studied through QSAR modeling software (T.E.S.T.) recommended by USEPA (2012). It was found that combined form of phytochemicals in plant extracts of *Phyllanthus* sp. act as non-toxic and/or antimutagenic (Kumar et al., 2002) but individual phytochemical such as alkaloid, lignin, phytosterol, stanol etc. have showed potent toxicity, carcinogenicity and mutagenicity (Normen et al., 2001; Ifere et al., 2010; Bode and Dong, 2015).

The present QSAR modeling work is more relevant in future through experimental and predictive study by using other 2D and 3D based prediction software to know the mechanisms of toxicity and mutagenicity for each natural chemical, which found in a combined form phytochemicals in extract of different parts of *P. niruri* for therapeutic usage.

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