Chapter-2

Experiment-V

Identification of analogous chemicals for anthraquinone, β-sitosterol and quercetin found in *Desmostachya bipinnata, Parthenium hysterophorus and Alternanthera sessilis* by using Chem*ID* plus and and their predictive toxicity testing using Toxicity Estimation Software Tool :- A software approach.

Introduction:-

Using conventional experimental toxicological methods to know a large family of allelochemicals, is time consuming and costly. The United States Environmental Protection Agency (USEPA) have decided to move away from animal studies, in assessing of chemicals for safety of the environment. Computational methods offer an attractive alternative to expensive experimental studies, with the potential for several studies on many allelophytochemicals (Sullivan *et al.*, 2014).

Computational tools evaluate numerous compounds for multi dimensional toxicological end-points. This screening method, using software is easy, highlighting compounds for which experimental evaluation is recommendable. It produces a system for predicting the allelochemical toxicity and/or mutagenicity. This system can also give additional information, by identifying genotoxic properties in a fast and convenient way on the basis of the simple chemical structure. These models integrate knowledge from (chemistry, toxicology and computer science) that together show a possible relationship by predicting the property of a chemical from its structure, without direct experimental measurement (Lo Piparo *et al.*, 2006).

Mutagenicity is directly an indicative of toxic potential of an allelochemical. Scientists have documented that soil plays an important role as the biological environment where allelochemicals have easily been either detoxified or toxified through microbial conversion (Inderjit and Weiner, 2001; Ohno, 2001). The microbial

activities in the soil is based on moisture content- the main edaphic factor that helps to enhance soil activity by microorganisms (Reinhardt *et al.*, 1996; Rizvi and Rizvi, 1992).

This is because all allelochemicals are transported through water, which serves as a solvent and carrier of leachates from all aerial parts of the plant in the soil.

Interestingly, some of these bioactive allelochemicals may act on important crop plants such as cereals, legumes,pulses etc. by the presence of natural toxins or mutagens (Adler and Chase, 2007; Islam and Kato-Noguchi, 2013; Leather, 1983).

Among several weeds, *Alternanthera sessilis* (Amaranthaceae), a profusely growing weed species in West Bengal was selected because it is ecologically well adapted with several co-evolving ecotypes i.e. *A. tenella, A. ficoidea* etc, stress tolerant nature (Hunt *et al.*, 1987). There are many allelochemicals in the roots, stem and leaves of *A. sessilis*, *A. tenella, A. ficoidea etc* which have been well documented in literatures out of which anthraquinone – a shikimic acid derivative, β-sitosterol an alkaloid containing six isoprene units obtained from mevalonate pathway and quercetin a phenol obtained from cinnamic acid derivatives and their related compounds have been well studied by researchers (Brown and Brown, 1976; Materska, 2008; Wolfreys and Hepburn, 2002). Interestingly *Desmostachya bipinnata* which is even found to suppress the growth of *Parthenium hysterophorus* (Javaid *et al.*, 2005) both contain anthraquinone, β-sitosterol and quercetin in addition to several phenolic acids and sesquiterpene lactones.

(Serafimova *et al.*, n.d.) 2010, documented Ames mutagenicity prediction with an accuracy of about 70%-75%. Mutagenic activities are based on base-pair substitutions or

frame-shift mutations that can only occurr in strains of *Salmonella typhimurium* which depend on histidine-rich medium for any chemical to be tested (Ames, 1979). The concept is reverse mutations caused by the functional ability of the bacterial strain to synthesize histidine dependent colony growth on the histidine deficient medium during mutagens exposure, called as revertant colony (USEPA, 2012). The test chemical is classified Ames mutagenic positive when it induces revertant colony growth in any one of out of five strains (TA97, TA98, TA100, TA102 and TA104) often used and vice versa for mutagenic negative (Mortelmans and Zeiger, 2000).

The T.E.S.T. (Toxicity Estimation Software Tool) is a 2-D, Q.S.A.R. (Quantitative Structure–Activity Relationship)- modeling software, which helps in mutagenicity prediction for major organic compounds. In the T.E.S.T., a dataset of 6512 chemicals was incorporated from several sources and from Toxicity Benchmark (Hansen *et al.*, 2009). The final dataset have consisted of 5743 chemicals where excluding mixtures, ambiguous organic materials, salts, and compounds having unavailable Chemical Abstracts Service-CAS numbers.

For external statistical validation, the consensus method gives the best prediction accuracy, which is based on concordance value and prediction specificity (USEPA, 2012). The predicted toxicity is simply the average of the predicted values from the above mentioned QSAR methodologies (Zhu *et al.*, 2009). In addition this method provides very broad prediction coverage because several methods with slightly different applicability domains are used to make a prediction (Ruiz *et al.*, 2012).

The present study thus aims to predict the mutagenicity of similar analogues of the three common phytoallelochemicals viz. anthraquinone, β-sitosterol and quercetin present in *Desmostachya bipinnata, Parthenium hysterophorus and Alternanthera sessilis* by using *ChemID plus* and T.E.S.T. software and corroborate it with the obtained experimental value and validate the data .

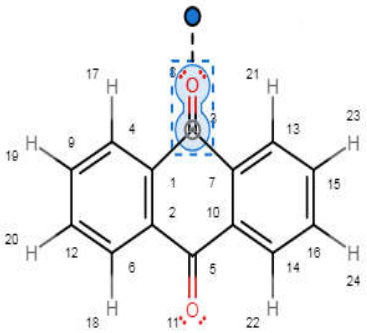
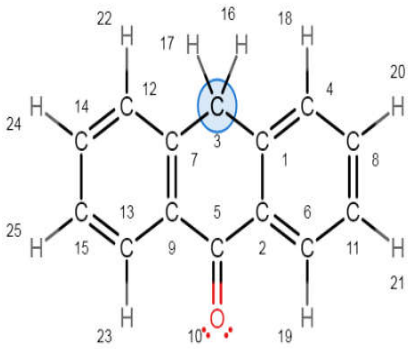
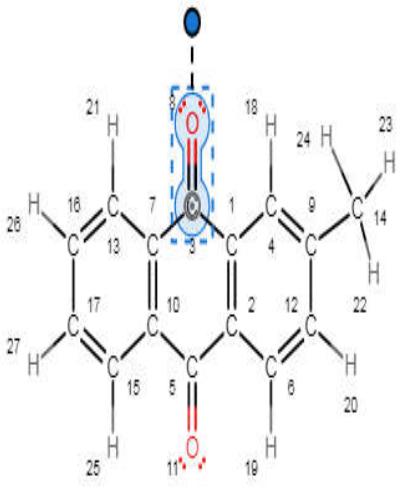
2. MATERIALS AND METHODS

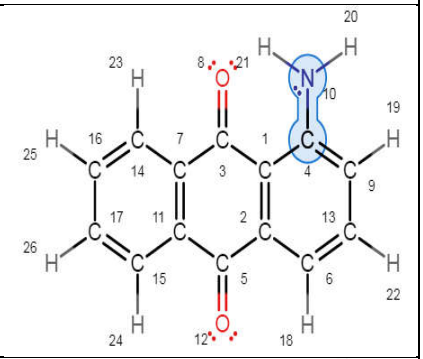
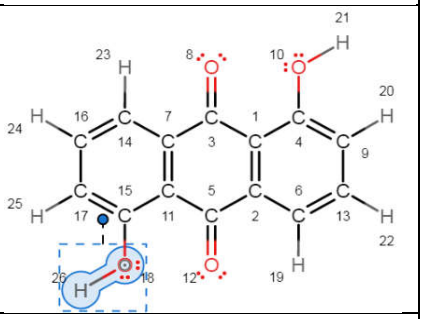
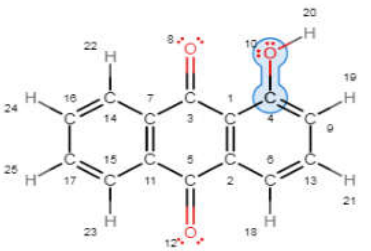
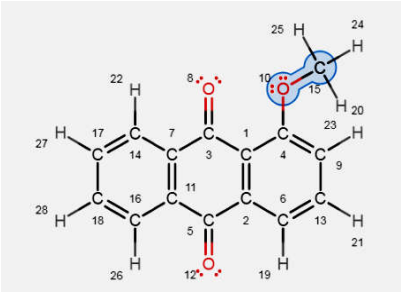
In the present study, three common phytochemicals viz. anthraquinone, β-sitosterol and quercetin, commonly available in *D.bipinnata, P.hysterophorus and A. sessilis* and nine structurally similar compounds for each phytochemical were selected as shown in tables were selected for in silico prediction of Ames Mutagenicity through T.E.S.T. software Ver 4.1.Nine analogous chemicals For each individual phytoallelochemical were selected on the basis of similar molecular structure and they were tabulated along with (CAS) no. obtained from ChemID*plus*- which is a free, web search system that provides access to the structure and nomenclature authority files used for the identification of chemical substances cited in National Library of Medicine (NLM) databases, including the Toxicology Data Network (TOXNET®) system.

Thirty chemicals were studied for toxicity. These are predicted either mutagenic(+ve) or mutagenic(-ve), through Ames Test. The T.E.S.T. estimates mutagenicity using several QSAR methodologies like Hierarchical clustering, FDA-where prediction for each test chemical is made using a new model that is fit to the chemicals that are most similar to the test compound.

Each model is generated at runtime, nearest neighbor and a consensus method- which is average of the predicted toxicities from each of the above QSAR methodologies, also obtained in present software based on the applicability domain for individual method (Zhu *et al.,* 2009). Particular chemical structure can easily be observed after entering CAS no. in appropriate text box of T.E.S.T. software interface to obtain predictive toxicity data. All the experimental data were also gathered through same. The Ames test has confirmed frameshift mutations or substitutions in base-pair and can only be simulated for any test chemical when exposed to *Salmonella typhimurium* of histidine-dependent strains,(USEPA, User Guide,2012).

Table 29(a). Identification of similar allelophytochemicals analogous to Anthraquinone present in *Desmostachya bipinnata, Parthenium hysterophorus*, *Alternanthera sessilis.*

Sl. No.	Parent chemical with molecular formula*	Similar chemical with molecular formula*	CAS No*	Structure*
1.	Anthraquinone (C_{14}-H_8-O_2)	---------	**84-65-1**	
2		Carbothrone (C_{14}-H_{10}-O)	**90-44-8**	
3		2-Methylanthraquinone (C_{15}-H_{10}-O_2)	**84-54-8**	

Sl. No.	Parent chemical with molecular formula*	Similar chemical with molecular formula*	CAS No*	Structure*
4		**1-Aminoanthraquinone (C_{14}-H_9-N-O_2)**	**82-45-1**	
5		**1,5-Dihydroxyanthraquinone (C_{14}-H_8-O_4)**	**117-12-4**	
6		**1-Hydroxy anthraquinone (C_{14}-H_8-O_3)**	**129-43-1**	
7		**9,10-Anthracenedione, 1-methoxy- (C_{15}-H_{10}-O_3)**	**82-39-3**	

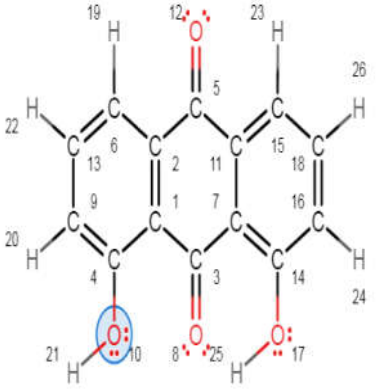
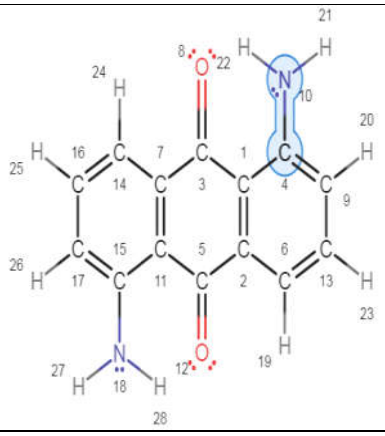
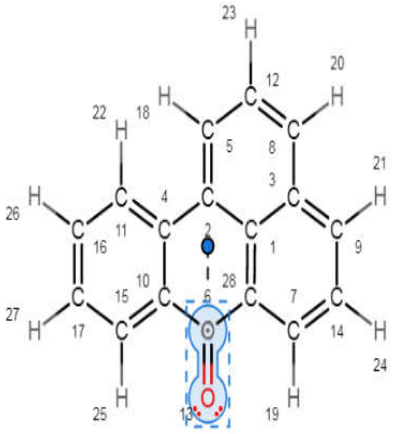
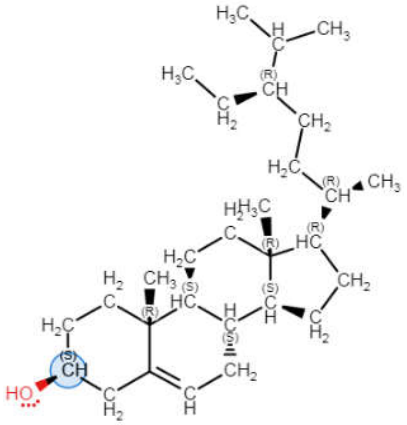
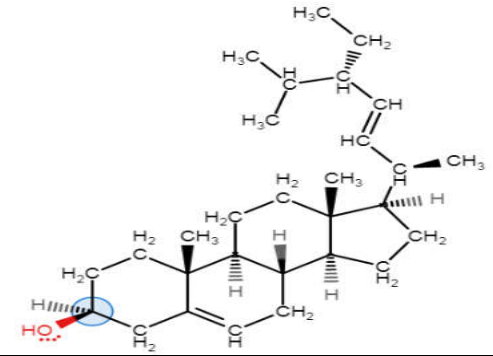
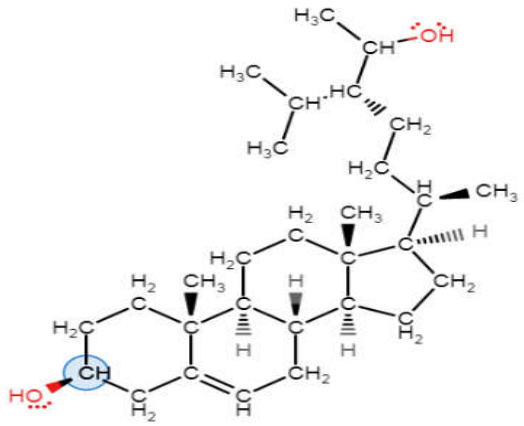
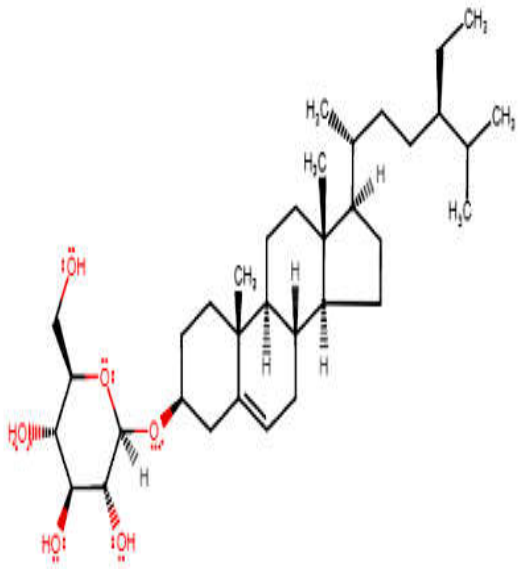
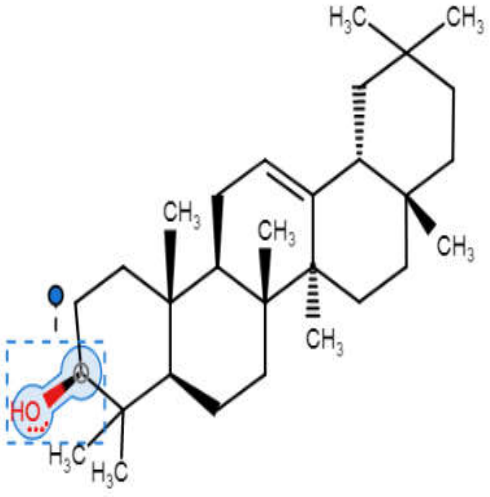
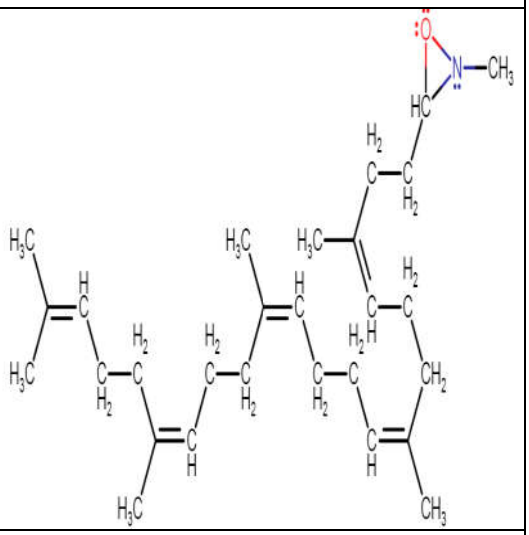
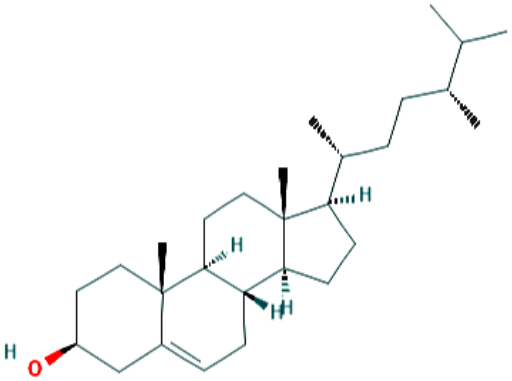
Sl. No.	Parent chemical with molecular formula*	Similar chemical with molecular formula*	CAS No*	Structure*
8		**Chrysazin (C_{14}-H_8-O_4)**	**117-10-2**	
9		**1,5-Anthraquinonyldiamine (C_{14}-H_{10}-N_2-O_2)**	**129-44-2**	
10		**Naphthanthrone (C_{17}-H_{10}-O)**	**82-05-3**	

Table 30(a). Identification of similar allelophytochemicals analogous to β-sitosterol present in *Desmostachya bipinnata, Parthenium hysterophorus*, *Alternanthera sessilis.*

Sl. No.	Parent chemical with molecular formula*	Similar chemical with molecular formula*	CAS No*	Structure*
1.	β-sitosterol ($C_{29}H_{50}O$)	----------	**83-46-5**	
2		Stigmasterol (C_{29}-H_{48}-O)	**83-48-7**	
3		Stigmast-5-ene-3beta,28-diol ($C_{29}H_{50}O_2$)	**53517-53-6**	

Sl. No.	Parent chemical with molecular formula*	Similar chemical with molecular formula*	CAS No*	Structure*
4		**Sitogluside** ($C_{35}H_{60}O_6$)	**474-58-8**	
5		**β-Amyrin** ($C_{30}H_{50}O$)	**559-70-6**	

Sl. No.	Parent chemical with molecular formula*	Similar chemical with molecular formula*	CAS No*	Structure*
6		Squalene N-methyl-oxaziridine ($C_{28}H_{47}NO$)	126267-95-6	
7		Campesterol (C_{28}-H_{48}-O)	474-62-4	

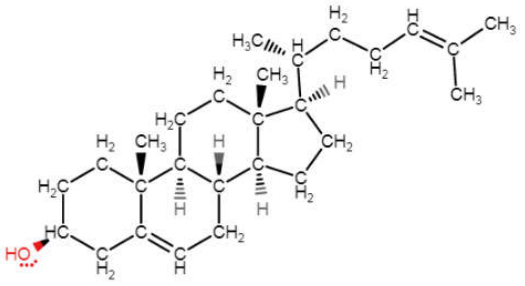
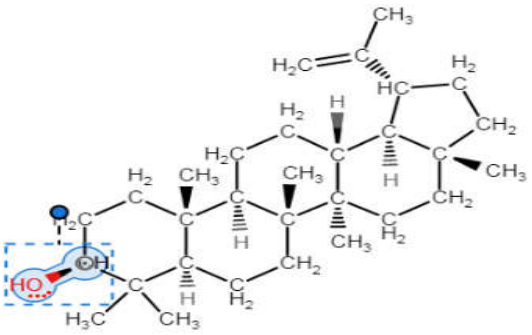
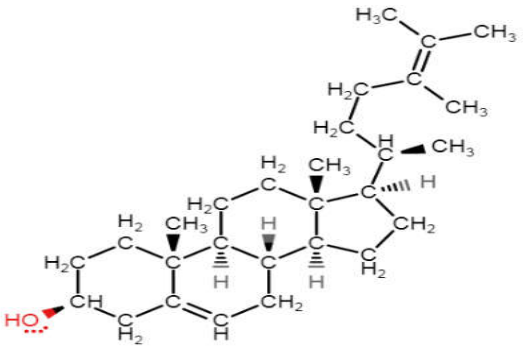
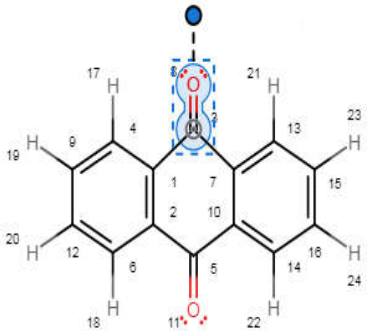
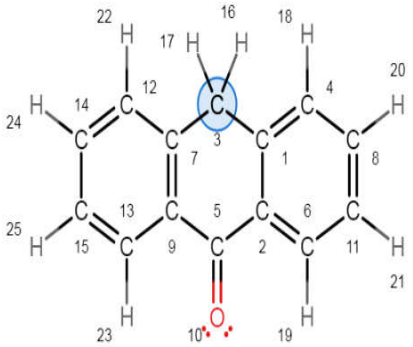
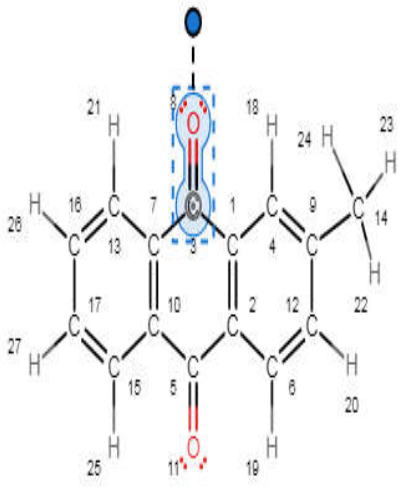
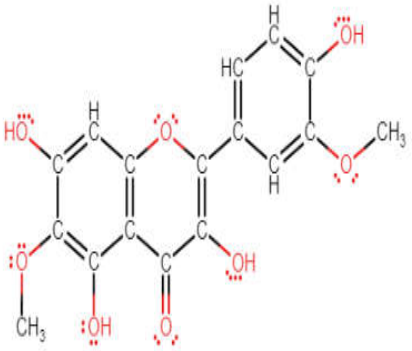
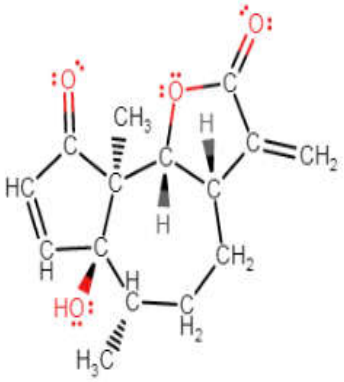
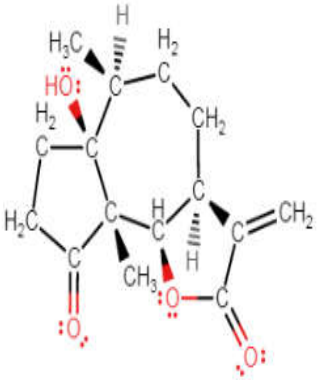
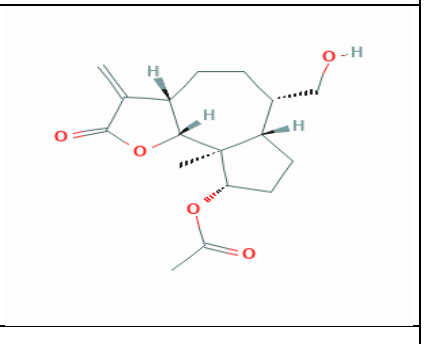
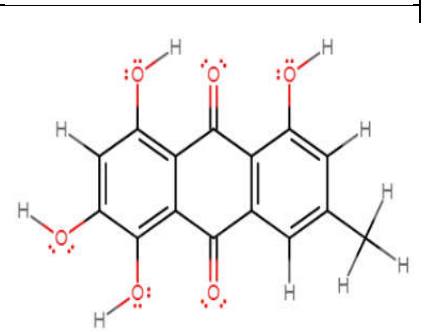
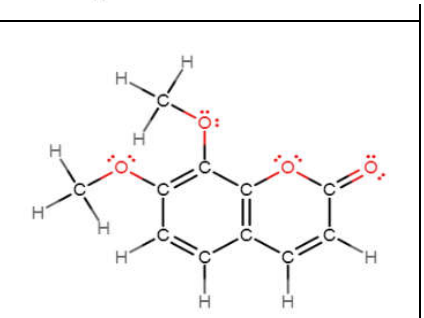
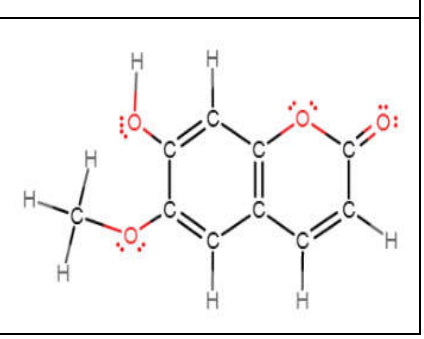
Sl. No.	Parent chemical with molecular formula*	Similar chemical with molecular formula*	CAS No*	Structure*
8		**Desmosterol ($C_{27}H_{44}O$)**	**313-04-2**	
9		**Lupeol (C_{30}-H_{50}-O)**	**545-47-1**	
10		**24- Methyl-desmosterol ($C_{28}H_{46}O$)**	**20780-41-0**	

Table 31(a). Identification of similar allelophytochemicals analogous to Quercetin present in *Desmostachya bipinnata, Parthenium hysterophorus*, *Alternanthera sessilis.*

Sl. No.	Parent chemical with molecular formula*	Similar chemical with molecular formula*	CAS No*	Structure*
1.	Quercetin (C_{15}-H_{10}-O_7)	---------	**117-39-5**	
2		Quercetin-3-glucoside **($C_{21}H_{19}O_{12}$)**	**90-44-8**	
3		Kaempferol (C_{15}-H_{10}-O_6)	**520-18-3**	

Sl. No.	Parent chemical with molecular formula*	Similar chemical with molecular formula*	CAS No*	Structure*
4		**Quarcetagetin 3',6, dimethyl ether (C_{17}-H_{14}- O_8)**	**82-45-1**	
5		**Parthenin ($C_{15}H_{18}O_4$)**	**508-59-8**	
6		**Coronopilin (C_{15}-H_{20}-O_4)**	**129-43-1**	

Sl. No.	Parent chemical with molecular formula*	Similar chemical with molecular formula*	CAS No*	Structure*
7		Hysterin ($C_{17}H_{24}O_5$)	5492-09-1	
8		5-Hydroxyemodin ($C_{15}H_{10}O_6$)	20324-66-7	
9		7,8-Dimethoxycoumarin ($C_{11}H_{10}O_4$)	2445-80-9	
10		Scopoletin ($C_{10}H_8O_4$)	92-61-5	

Source: Chem*ID*plus- Tables-29(a),30(a),31(a).

 # All data from Toxicity Benchmark through T.E.S.T;

(+) = mutagenic positive; (⁻) = mutagenic negative

[Tables- 29(b), 30(b), 31(b)]

Table 29(b). Predictive mutagenicity of Anthraquinone and its similar compounds.

		Ames mutagenicity Prediction through T.E.S.T.		
Srl no	**Allelochemicals**	**Experimental value #**		**Predicted value**
1.	Anthraquinone	1.00 (+)		0.74 (+)
2.	Carbothrone	1.00 (+)		0.57 (+)
3.	2-Methylanthraquinone	0.00 (-)		0.76 (+)
4.	1-Aminoanthraquinone	1.00 (+)		0.96 (+)
5.	1,5-Dihydroxyanthraquinone	1.00 (+)		0.94 (+)
6.	1-Hydroxyanthraquinone	1.00 (+)		0.91 (+)
7.	9,10-Anthracenedione,1-methoxy	1.00 (+)		0.73 (+)
8.	Chrysazin	1.00 (+)		0.94 (+)
9.	1,5-Anthraquinonyldiamine	1.00 (+)		1.07 (+)
10.	Naphthanthrone	1.00 (+)		0.20 (-)

Table 30(b). Predictive mutagenicity of *β*-sitosterol and its similar compounds.

		Ames mutagenicity Prediction through T.E.S.T.		
Srl no	**Allelochemicals**	**Experimental value #**		**Predicted value**
1.	*β*-sitosterol	0.00		0.25 (-)
2.	Stigmasterol	1.00 (+)		0.57 (+)
3.	Stigmast-5-ene-3beta,28-diol	1.00 (+)		0.46 (+)
4.	Sitogluside	1.00 (+)		0.76 (+)
5.	β-Amyrin	1.00 (+)		0.64 (+)
6.	Squalene N-methyl-oxaziridine	0.00		0.06(-)
7.	Campesterol	0.00		0.43 (-)
8.	Desmosterol	1.00 (+)		0.69 (+)
9.	Lupeol	1.00 (+)		0 .87 (+)
10.	24- Methyl- desmosterol	1.00 (+)		0.30 (+)

Table 31(b). Predictive mutagenicity of Quercetin and its similar compounds.

		Ames mutagenicity Prediction through T.E.S.T.		
Srl no	**Allelochemicals**	**Experimental value #**		**Predicted value**
1.	Quercetin	1.00 (+)		0.55 (+)
2.	Quercetin-3-glucoside	1.00 (+)		0.52 (+)
3.	Kaempferol	1.00 (+)		0.38 (+)
4.	Quarcetagetin 3',6, dimethyl ether	1.00 (+)		0.61 (+)
5.	Parthenin	1.00 (+)		0.91 (+)
6.	Coronopilin	1.00 (+)		0.87(+)
7.	Hysterin	1.00 (+)		0.92 (+)
8.	5-Hydroxyemodin	1.00 (+)		0.75 (+)
9.	7,8-Dimethoxycoumarin	1.00 (+)		0 .81 (+)
10.	Scopoletin	1.00 (+)		0.78 (+)

DISCUSSION:

The predictive mutagenicity results clearly indicated that phytochemical anthraquinone and its related compounds were all mutagenic positive. All the experimental and predicted values were found mutagenic positive - Table 29(b).

Other phytochemical β-sitosterol showed mutagenic negative in predicted values along with two other compounds namely- Squalene N-methyl-oxaziridine and Campesterol, but the remaing seven related compounds were mutagenic positive after prediction by using T.E.S.T as can be seen from Table-30(b).

In case of Quercetin, both experimental and predicted values were obtained mutagenic positive while in related analogous compounds also all showed positive mutagenecity. It was observed that Parthenin, Hysterin, Coronopilin, 7,8-Dimethoxycoumarin,Scopoletin and 2-hydroxyemodin, were predicted to be extremely high mutagenic positive as evident from the values in Table31(b).

It has been documented that the secondary metabolite anthraquinone and its similar compounds as mentioned in the results section were potent mutagen on *S. typhimurium* in experimental study (Brown and Brown, 1976; Tamaro *et al.*, 1975). The present prediction data were also obtained mutagenic positive. and much above (0.50) which is the least value to be mutagenic positive. The majority of natural anthraquinones and its analogues are weakly acidic due to the influence of electronic attraction of the carbonyl group on hydroxyl. The hydrogen bond between carbonyl and adjacent hydroxyl ion hampers the dissociation of the hydroxyl proton. Therefore, they dissolve only in

Sodium hydroxide solution. Thus an alkaline soil pH is a stress factor that can promote their dissolution in the soil and their consequent absorption into surrounding roots of several other plants inhibiting germination of seeds and formation of primary roots (Romagni *et al.*, 2004).

The allelochemical, quercetin also showed positive mutagenecity in purified form to *Salmonella typhimurium* (Bjeldanes and Chang, 1977; Stoewsand *et al.*, 1984) and prediction by T.E.S.T. reported mutagenic positive with a value of (0.55) exceeding the barrier limit of (0.50) when studied without known mutagen (Banerjee and Talapatra, 2015; Borges de Melo *et al.*, 2010; Edenharder and Tang, 1997), which supports the present findings.

In soil, phosphate depletion occurred when hydroxyemodin was released by weeds (Inderjit and Nishimura, 1999). It has been emphasized that a series of hydroxyemodin analogues are reported to inhibit seed germination and primary root development (Romagni *et al.,* 2004). Among all the phytochemicals, β-sitosterol was obtained non-mutagen in both experimental and prediction data with a value of (0.25).

Similar observations have been found in other research works in relation to several pesticides that few compounds have no mutagenicity values for experimental results but same compounds have negative mutagenecity for predicted values by using T.E.S.T. (Saini *et al.*, 2014).

The present study is undertaken with a curiosity that whether substitutional alterations occur on their position in producing its related analogues in these secondary metabolites viz. anthraquinone, β-sitosterol and quercetin of *D. bipinnata, P. hysterophorus, A. sessilis* due to environmental stress in presence of other elementary toxins exposed in the soil. Water as solvent either dissolves or changes their configuration, that is why it is worthwhile knowing what is the ultimate fate of available phytochemical and its molecular configuration in soil?

4. CONCLUSIONS

In conclusion, a pertinent question arises – regarding the possibilities of conversion or substitution in the aromatic ring of the allelochemicals when these release in soil from these three weed plants. There is documented proof that the plant allelopathy is fully dependent upon several biotic and abiotic factors and degradation can be achieved by soil microorganisms (Inderjit and Weiner, 2001; Ohno, 2001).

Solar radiation, gradients in temperature, types of soil and parameters like salinity, moisture content, nutrients, insects and diseases in the soil are several other causes of allelopathy (El-Darier *et al.*, 2014; Li *et al.*, 2010; Soltys *et al.*, 2013).

Generally phenolic extracts have proved to confirm DNA strand breaks and thus contributing significantly to genotoxicity and mutation. Allelochemicals like quercetin, parthenin, hysterin inhibit topoisomerase I and II enzyme, which has capacity to interfere with the replication and transcription ,inhibiting the process of the re-joining of ds-DNA

breaks and increasing the formation of DNA- enzyme complexes which are cleavable (Matthews *et al.*, 2006).

The present study might be a possible indication in future to detect metabolic pathway and also to know the mechanism of allelochemical formation in the three weeds under study in presence of organic and/or elementary toxins in soil. It should be investigated in future that whether the studied allelochemicals and their analogues increase the toxic impact on other weeds as bioherbicides. The present study was carried out with (T.E.S.T.) but suggested to validate with other available softwares.