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Research Article

PHYTOCHEMISTRY AND BIOINFORMATICS APPROACH FOR THE EVALUATION OF MEDICINAL PROPERTIES OF THE HERB, *EXACUM BICOLOR* ROXB.

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ABSTRACT
Exacum bicolor Roxb. (Gentianaceae) is a phytochemically unexplored traditional medicinal herb, generally distributed in the grasslands of northern Kerala during July-October. The present study through GC-MS analysis revealed the presence of six phytochemical compounds of medicinal importance (two compounds of polyphenolic group *viz*. 7'-Chloro-3'-(2, 4 dichlorophenyl)-3',4'-dihydrospiro(1, 3- dioxolane- and a'-D- Galactopyranoside, methyl 2,6- bis-0-(trimethylsilyl) -, cyclic butylboronate, two compounds of alkaloid group *viz*. 1, 16-Cyclocorynan-16-carboxylic acid, 17-(acetyloxy)-19,20-didehydro-10-methoxy-, methyl ester,(16.xi., 19E)- and 4 – (4 – Chlorophenyl)- 5 – morpholin - 4 - yl- thiophen -2- carboxylic acid, ethyl ester, one compound of glycoside group, a'-D- Galactopyranoside, methyl 2,3- bis-0-(trimethylsilyl) -, cyclic phenylboronate and one compound of steroid group, 9,19 – Cycloergostan – 3 – ol – 7 – one , 4 , 14 – dimethyl –) in addition to number of other compounds. In bioinformatics approach, by using the software, Prediction Activity Spectra for Substances (PASS), molecular formula, pharmacological effects and drug likeness were determined for all the six compounds scientifically which confirm the traditional usage of *Exacum bicolor*.
KEY WORDS- *Exacum bicolor*, Medicinal plant, Phytochemistry, PASS,

INTRODUTION

The complex and diverse chemical structures of natural compounds provide the basis for modulation of different biological targets[1]. Multitargeted actions of natural compounds could lead to additive/synergistic or antagonistic effects[2]. Since there are several thousands of known pharmacological targets and natural products exhibit pleiotropic action interacting with multiple targets, computer-aided methods could be extremely useful for the evaluation of natural products[3]. *Exacum bicolor* Roxb. (Gentianaceae), generally distributed in Northern Kerala state of India in savannas and grasslands of Western Ghats during July-October is priscribed by the local traditional healers for various ailments like fever, eye and skin diseases, urinary disorders and malaria. The whole plant extract is used as tonic and stomachic[4&5]. Being bitter in taste local people take it as herbal remedy for diabetes and skin disorders also[6]. Despite, these diverse therapeutic uses so far no scientific work has been carried out in this species. Hence, to determine the active principal compounds of *E. bicolor,* phytochemical analysis and prediction of molecular formula and drug likeness by using the computer programme, Prediction Activity Spectra for Substances (PASS) were carried out.

MATERIALS AND METHODS

Collection of the plant material

The study species, *E. bicolor* collected from the grasslands of Kannur district of Kerala was dried for 20 days at room temperature and powdered for further analysis.

Extraction

100 g powdered whole plant material was exhaustively extracted by using methanol solvent in soxhlet apparatus for 24 hr in order to get maximum yield of soluble compounds[7]. The crude extract was filtered and concentrated under vacuum and controlled temperature with a rotary evaporator and residues were freeze dried. The extract was stored at -8°C in deep freezer until further use.

Gas Chromatography - Mass Spectrometry (GC-MS)

Five ml of methanol extract was evaporated to dryness and reconstituted in 1 µl methanol. The extracts were then subjected to GC-MS analysis. Chromatographic separation was made with Jeol gc matell Front instrument with Hp 5 mr column (30 m x 0.32 mm, 0.033 µm film thickness). Heating programs were executed from 50 to 280^{o} C by using helium as a carrier gas with the flow rate of 1 ml/min in the split mode (1:2). An aliquot (1 µl) of oil was injected into the column with the injector heater at 220^{o} C[8].

Analytical conditions

Injection temperature at 220° C, oven temperature at 50 – 280° C, interface temperature at 280° C and quadruple temperature at 280° C were maintained. Injection was performed in split mode.

Data analysis

The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV, and the detector was operated in scan mode from 50 to1000 atomic mass units (amu). Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and NIST[9&10].

Prediction Activity Spectra for Substances (PASS)

This computer system can predict biological activity based on structural formula of a chemical compound. The PASS approach is based on the suggestion, Activity = Function (Structure). Thus, "comparing" structure of a new substance with that of the standard biologically active substances, it is possible to find out whether a new substance has a particular effect or not. PASS estimates the probabilities of a particular substance's belonging to the active and inactive sub-sets from the SAR Base (Structure-Activity Relationships Base) **[11&12]**.

External files of substances

PASS uses SDfile (.sdf) or MOLfile (.mol) formats[13] as an external source of structure and activity data to prepare both SAR Base and the set of substances to be predicted. SDfiles can be exported either from ISIS/Base 2.0+ (MDL Information Systems, Inc.) or from another molecular editor which has the option of SD file's export. MOLfiles can be prepared by ISIS/Draw. Molecular properties and 3D structure of a compound were determined by using .sdf format which is obtained from Pubchem database (NCBI)[14]. The .mol generates 3D images using ArgusLab[15].

Algorithm of prediction

The result of prediction is returned in the form of a table containing the list of biological activity with the appropriate probability values (i.e.) the values defining the likelihood for a given activity type to be either revealed (Pa) or not revealed (Pi) for each activity type from the predicted biological activity spectrum. Their values vary from 0.000 to 1.000. Only those activity types for which Pa > Pi are considered possible**[16]**.

RESULTS AND DISCUSSION

The GC-MS studies in the methanolic extract of *E. bicolor* showed the presence of rich variety of phytochemical compounds (Table 1 and Figures 1-7). The constituents are belonging the groups as described below: two polyphenolic compounds of (7'-Chloro-3'-(2, 4 dichlorophenyl)-3',4'-dihydrospiro(1, 3- dioxolane-, and a'-D- Galactopyranoside, methyl 2,6- bis-0-(trimethylsilyl) -, cyclic butylboronate), two compounds of alkaloids (1, 16- Cyclocorynan-16-carboxylic acid, 17-(acetyloxy)-19,20-didehydro-10-methoxy-, methyl ester,(16.xi., 19E)- and 4 – (4 – Chlorophenyl)- 5 – morpholin - 4 - yl- thiophen -2- carboxylic acid, ethyl ester), one compound of glycosides (a'-D-Galactopyranoside, methyl 2,3- bis-0-(trimethylsilyl) -, cyclic phenylboronate,) and one compound of steroids (9,19 – Cycloergostan – 3 – ol – 7 – one , 4 , 14 – dimethyl –). It has been reported already that these phytochemicals belonging to different secondary metabolites such as alkaloids, glycosides, phenols and steroids have high medicinal activity and are used for many pharmacological activities including anticancer, anti-inflammatory and antiaging properties[17-19]. In an another Gentianaceae member, *Gentiana asclepiadea,*[20] identified rich variety of secondary metabolites including certain essential oils which may be attributed as a factor for its medicinal properties.

In order to find out the structure and specific activity of these compounds it is under gone for prediction of activity by using PASS software. Type of biological activity predicted by PASS includes the pharmacological effects, toxicity, molecular mechanisms of and drug likeness of compounds are presented in Table 2. The high drug likeness for the two compounds, 9,19 – Cycloergostan – 3 – ol – 7 – one , 4 , 14 – dimethyl – and 1, 16- Cyclocorynan-16-carboxylic acid, 17-(acetyloxy)-19,20-didehydro-10-methoxy-, methyl ester,(16.xi., 19E)- are determined to be 0.993 and 0.956 respectively which proved the high probability of the plant, *E. bicolor* as a drug. The anti-hypertensive and vasodilator activity of the compound 1, 16- Cyclocorynan-16-carboxylic acid, 17-(acetyloxy)-19,20-didehydro-10-methoxy-, methyl ester,(16.xi., 19E)- predicted the pharmacological effect of this compound. In the similar fashion,[21] predicted thirty molecular mechanisms of action of compounds which cause antihypertensive effect on basis of the structural formulae by the computer programme PASS.

Pa and Pi values of each activity was also studied using PASS (Table 3).The predicted spectra of biological activity also express the side effects and toxicity of the compounds. Attentions have to be paid to both undesirable side effects and toxicity. PASS predicted the embryotoxicity, carcinogenicity and teratogenicity of the compounds, 7'-Chloro-3'-(2, 4 dichlorophenyl)-3',4'-dihydrospiro(1, 3- dioxolane- and 9,19 – Cycloergostan – 3 – ol – 7 – one , 4 , 14 – dimethyl – which is suggested to nullify by developing appropriate strategies.

Moreover, it was shown that the algorithm used in PASS can successfully be applied to discriminating the so-called 'drug-like' compounds from 'drug-unlike' substances[22]. Similar studies were carried out earlierly for various compounds[22-25] for the prediction of the biological activities.

CONCLUSION

GC-MS analysis isolates the six different compounds of medicinal importance from the methanol extract of the species, *E. bicolor*. Prediction of biological activity of these compounds by using the PASS software was successful to some extent. The reports of the study confirm the traditional medicinal usage of *E. bicolor* for various ailments in northern Kerala. However large data must be generated through pharmacognostic studies before going commercialization.

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Table-1 Phytochemical compounds of the methanolic extract of *Exacum bicolor* using GC-MS analysis

S.No	Phytochemical compound	Molecular formula	Retention time/min.	Molecular weight (m/z)
1	7'-Chloro-3'-(2, 4 dichlorophenyl)-3',4'-dihydrospiro(1, 3- dioxolane-	$C_{12}H_{15}Cl_3O_3S$	16.76	409
2	1, 16- Cyclocorynan-16-carboxylic acid, 17-(acetyloxy)-19,20-didehydro-10-methoxy-, methyl ester,(16.xi., 19E)-	$C_{24}H_{28}N_2O_5$	17.68	424
3	4 – (4 – Chlorophenyl)- 5 – morpholin - 4 - yl- thiophen -2- carboxylic acid, ethyl ester	$C_{17}H_{18}ClNO_3S$	20.05	351
4	a'-D- Galactopyranoside, methyl 2,6- bis-0-(trimethylsilyl) -, cyclic butylboronate	$C_{17}H_{37}BO_6SI_2$	21.13	357
5	a'-D- Galactopyranoside, methyl 2,3- bis-0-(trimethylsilyl) -, cyclic phenylboronate	$C_{18}H_{30}BO_2SI_2$	21.81	411
6	9,19 – Cycloergostan – 3 – ol – 7 – one , 4 , 14 – dimethyl –	$C_{30}H_{50}O_2$	18.9	424

Table-2 Predicted activities of compounds identified from GC- MS analysis in *E. bicolor* by using PASS.

Compound	Molecular formula	Hydrogen bond donor	Hydrogen bond accepter	Activity			Drug likeness
				Pharmacological effects	Side effects and toxicity	Molecular mechanisms	
7'-Chloro-3'-(2, 4 dichlorophenyl)-3',4'-dihydrospiro(1, 3- dioxolane-	$C_{12}H_{15}Cl_3O_3S$	0	3	Nil	Embryotoxic Carcinogenic Teratogen	5Hydroxytryptamine 2 antagonist Neuropeptide Y antagonist Calcium channel antagonist Calcium antagonist	0.412
1, 16- Cyclocorynan-16-carboxylic acid, 17- (acetyloxy)-19,20-didehydro-10-methoxy-, methyl ester,(16.xi., 19E)-	$C_{24}H_{28}N_2O_5$	0	6	Vasodilator Antihypertensive	Nil	Alpha 2 adrenoreceptor antagonist Nitric oxide agonist Adrenaline antagonist Nitric oxide donor Calcium channel antagonist	0.956

Table-3 Predicted Pa and Pi values for the GC-MS identified compounds of *E. bicolor* by using PASS.

Compound	Molecular formula	Hydrogen bond donor	Hydrogen bond accepter	Activity			Drug likeness
				Pharmacological effects	Side effects and toxicity	Molecular mechanisms	
4 – (4 – Chlorophenyl)- 5 – morpholin - 4 - yl- thiophen -2- carboxylic acid, ethyl ester	$C_{17}H_{18}ClNO_3S$	0	4	Nil	Nil	Calcium channel antagonist Guanylate cyclase stimulant Nitric oxide agonist	0.153
a'-D- Galactopyranoside, methyl 2,6- bis-0-(trimethylsilyl) -, cyclic butylboronate	$C_{17}H_{37}BO_6SI_2$	0	6	Nil	Nil	Nil	0.295
9,19 – Cycloergostan – 3 – ol – 7 – one , 4 , 14 – dimethyl –	$C_{30}H_{50}O_2$	1	2	Nil	Embryotoxic Teratogen	Nil	0.993

S.No.	Compounds	Activity		Pa	Pi
1.	7'-Chloro-3'-(2, 4 dichlorophenyl)-3',4'-dihydrospiro(1,3-dioxolane-	Pharmacological effects	Nil	-	-
		Side effects and toxicity	Embryotoxic Carcinogenic Teratogen	0.404 0.305 0.228	0.071 0.089 0.201
		Molecular mechanisms	5Hydroxytryptamine 2 antagonist Neuropeptide Y antagonist Calcium channel antagonist Calcium antagonist	0.804 0.254 0.127 0.080	0.005 0.067 0.093 0.067
2.	1,16- Cyclocorynan-16-carboxylic acid, 17-(acetyloxy)-1 9,20-didehydro-10-methoxy-, methyl ester,(16.xi., 19E)-	Pharmacological effects	Vasodilator Antihypertensive	0.514 0.244	0.053 0.086
		Side effects and toxicity	Nil	-	-
		Molecular mechanisms	Alpha 2 adrenoreceptor antagonist Nitric oxide agonist Adrenaline antagonist Nitric oxide donor Alpha adrenoreceptor antagonist Calcium channel antagonist	0.511 0.279 0.134 0.073 0.111 0.123	0.011 0.205 0.073 0.021 0.077 0.099
3.	4 – (4 – Chlorophenyl)-5 – morpholin - 4 - yl-thiophen -2- carboxylic acid, ethyl ester	Pharmacological effects	Nil	-	-
		Side effects and toxicity	Nil	-	-
		Molecular mechanisms	Calcium channel antagonist Guanylate cyclase stimulant Nitric oxide agonist	0.324 0.140 0.116	0.121 0.064 0.108
4.	a'-D-Galactopyranoside, methyl 2,6-bis-0-(trimethylsilyl) -,cyclic butylboronate	Pharmacological effects	Nil	-	-
		Side effects and toxicity	Nil	-	-
		Molecular mechanisms	Nil	-	-
5.	9,19 – Cycloergostan – 3 – ol – 7 – one , 4 , 14 – dimethyl –	Pharmacological effects	Nil	-	-
		Side effects and toxicity	Embryotoxic Teratogen	0.370 0.358	0.084 0.102
		Molecular mechanisms	Nil	-	-

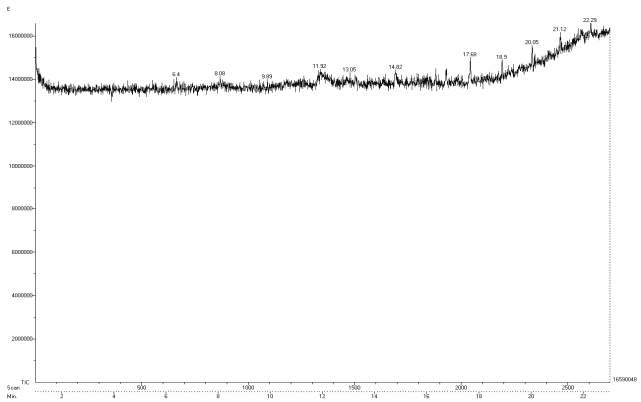

Figure.1. Gas chromatogram of the methanolic extract of *Exacum bicolor*.

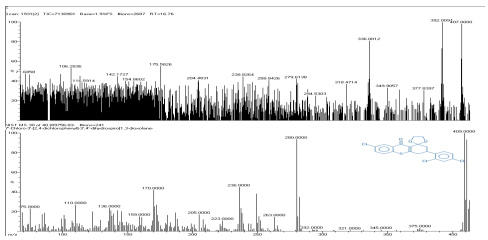

Fig. 2

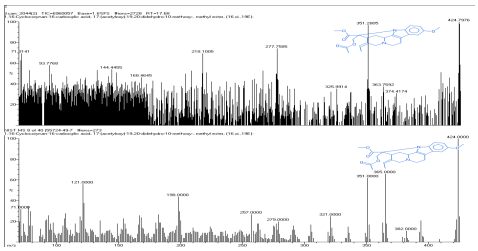

Fig. 3

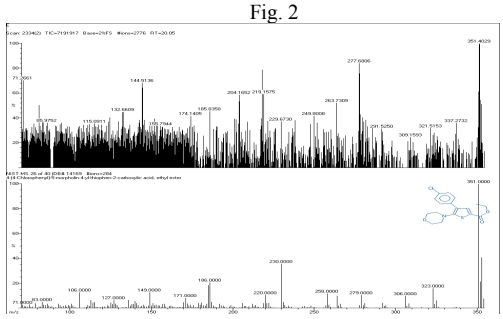

Fig. 4

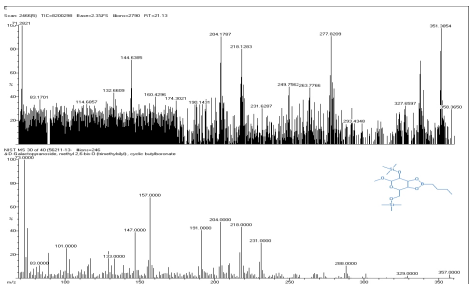

Fig. 5

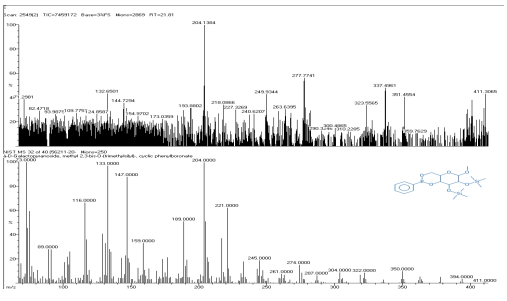

Fig.6

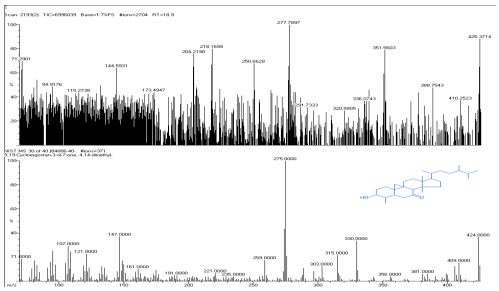

Fig.7

Figs. 2 -7. Mass spectra for compounds in methanolic extracts of *Exacum bicolor*

Fig. 2. 7'-Chloro-3'-(2, 4 dichlorophenyl)-3',4'-dihydrospiro(1, 3- dioxolane-

Fig. 3. 1, 16- Cyclocorynan-16-carboxylic acid, 17-(acetyloxy)-19,20-didehydro-10-methoxy-, methyl ester,(16.xi., 19E)-

Fig. 4. 4 – (4 – Chlorophenyl)- 5 – morpholin - 4 - yl- thiophen -2- carboxylic acid, ethyl ester

Fig. 5. a'-D- Galactopyranoside, methyl 2,6- bis-0-(trimethylsilyl) -, cyclic butylboronate

Fig. 6. a'-D- Galactopyranoside, methyl 2,3- bis-0-(trimethylsilyl) -, cyclic phenylboronate

Fig. 7. 9,19 – Cycloergostan – 3 – ol – 7 – one , 4 , 14 – dimethyl –)

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