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ISOLATION, PURIFICATION, CRYSTAL STRUCTURE DETERMINATION OF 1, 3-BENZOXAZOL-2(3H)-ONE FROM *CROSSANDRA INFUNDIBULIFORMIS* FLOWER EXTRACT AND ANTIMICROBIAL EVALUATION

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ABSTRACT

Crossandra infundibuliformis (Acanthaceae) is referred as "Fire cracker" plant, our present study is focused on the purification, characterization of bioactive compound from the flowers of *Crossandra infundibuliformis* and antimicrobial screening of the purified compound along with the flower extract of different solvent polarity. First step is the extraction of plant sample, followed by purification of the compound using column chromatography. In the chromatographic separation, one fraction was obtained in crystal form. The single crystal was subjected to X-ray Crystallography for structure determination and elucidated as 1,3 Benzoxazol-2(3H)-one. Antimicrobial efficacy of the *Crossandra infundibuliformis* flower extracts (with varying solvent polarity) and also with the purified compound is checked by Agar well diffusion method. To test the antibacterial activity, both gram positive (*Staphylococcus aureus and Mycobacterium Tuberclosis*) and gram negative (*Escherichia coli and Pseudomonas aeruginosa*) bacteria were used. Antifungal activity is checked by using *Candida albicans* and *Aspergillus niger*. Results of Antimicrobial screening shows that all organisms used in this study is inhibited by the purified compound and flower extract in the solvent order (most to least) as methanol, ethyl acetate, chloroform, aqueous and petroleum ether.

KEY WORDS

1, 3-Benzoxazol-2(3H)-one, *Crossandra infundibuliformis*, Antimicrobial activity and X-ray Crystallography.

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INTRODUCTION

Plant compounds with medicinal value have been used over many years as drugs due to their safety towards human health. Secondary metabolites expelled out from the plants possess good defense mechanism against microorganism. Diverse activities have been reported by these metabolites such as anti-microbial, anti-cancer[1]. Among the organic compounds, heterocycles occupies an

important place due to their backbone or scaffolds which has wide application in pharmaceutical research. One of the main classes of heterocycles is the benzoxazole Figure No.1(a) which has a benzene ring fused with oxazole. It has a structure biosteroisomerism with nucleotides (guanine and adenine); thus can interact with the genetic material of the living system. Benzoxazolones have been reported in the Acanthaceae family and also in some of the vegetative crops such as maize, wheat. 1,3 Benzoxazol-2(3H)-one abbreviated as BOA (title compound) Figure No.1(b) was first reported in the rye seedlings in 1955. Later, in 1984 Murty *et al.*, reported this compound from *Acanthus ilicifolius* leaves. 6-Methoxy-2(3H)-benzoxazolone Figure No.1(c) was reported to have antimicrobial activity[2]. BOA shows good toxic effect toward the weeds attacking certain vegetable crops[3]. Benzoxazolone is referred as the template (commonly called as "privileged scaffold") with significant application in designing drug probes. This is due to the presence of functional groups and atoms in regard with ligand binding[4]. Some derivatives of BOA are available as drug in the market e.g. Benzolone which act as myorelaxant and Paraflex is a sedative analgesic. Derivatives of BOA exhibit various biological activities such as acetylcholine-sterase inhibitory activity[5], anticancer[6], antimicrobial[7], anti-HIV[8] and antipsychotic[9]. *Crossandra infundibuliformis* shown in Figure No.1(a) belongs to the family Acanthaceae. The leaf extract of C *infundibuliformis* is reported to have many properties such as aphrodisiac potential[10], antimicrobial activity[11], antioxidant activity[12], anticandidal[13] and larvicidal activity[14]. The flower part of this plant is reported to have wound healing activity[15]. Due to less exploration of the flower part, our previous study was focused on the phytochemical screening of flower extract which showed the presence of flavonoids, glycosides, terpenoids and phenolic compounds[16].

MATERIALS AND METHODS

Plant Collection and Extraction

The flowers of *Crossandra infundibuliformis* were collected from local market, Salem District, Tamil Nadu, India. The plant sample was identified and authenticated as *Crossandra infundibuliformis* belonging (Acanthaceae family) at the Botanical Survey of India, Coimbatore, India (No: BSI/SC/5/23/2013-14/Tech./705). The flowers were washed in clean water, shade-dried for 10 days and finely powdered. 30 grams of the flower powder was extracted sequentially with 150ml (each) of the solvent in the order petroleum ether, chloroform, ethyl acetate, methanol and aqueous by cold percolation method. Subsequently chromatographic separation was performed to obtain the pure compound. These extracts were used for antimicrobial screening.

Isolation and Purification of the compound

Column chromatography is a method to purify single compound from mixture of compounds. From the phytochemical screening, it is noticed that most of phytocompounds are present in methanolic extract. Thus, the methanolic extract was loaded in the column which was length 1m; packed with the silica gel as adsorbant. Initially the column was eluted with 100% petroleum ether followed by petroleum ether : chloroform, 100% chloroform, chloroform : ethyl acetate, 100% ethyl acetate, ethyl acetate : methanol, 100% methanol with varying polarity as (95:5,90:10,85:15,80:20,75:25, 70:30, 65:35,60:40,55:45,50:50,45:55,40:60,35:65,30:70,25 :75,280,15:85,10:90,5:95). The solvent in each elution was allowed to evaporate. One fraction/elution was obtained in crystal form, which was recrystallized to obtain a single crystal. The single crystal was then subjected to X-ray crystallographic studies and the structure of the compound was elucidated.

Microorganisms

For the antibacterial activity, bacterial strains of both gram positive (*Staphylococcus aureus and Mycobacterium Tuberclosis*) and gram negative (*Escherichia coli and Pseudomonas aeruginosa*)

were used. To screen the antifungal activity, *Candida albicans* and *Aspergillus niger* are used.

Preparation of media, inoculums and sample solution

Media preparation for the growth of microorganisms and inoculation was carried out as per the protocol given by Modiya et al., 2012. Required quantity of the purified compound was diluted in DMSO by serial dilution method. Both the solvent extracts and purified compound dilution were taken in various concentrations as 25µl, 50µl, 75µl, 100µl. Antibiotic disc, Ciprofloxacin (2 µg) is used as standard.

Antimicrobial screening

Antibacterial activity and antifungal activity of the plant extract and the purified compound is evaluated by comparing the value of inhibition zone against the standard drug value. Agar well diffusion method[17], a simple method and also easy measurement of inhibition zone is adopted to check these activities.

RESULTS AND DISCUSSION

Crystal structure determination using X-Ray Diffraction

Single crystal of the title compound shown in Figure No.1 was subjected to X-ray crystallographic studies. Bruker SMART CCD diffractometer, equipped with graphite monochromator was used to obtain the X-Ray Diffraction data of the single crystal of the title compound at room temperature with MoK_{α} radiation and the dimensions were recorded as 0.20 x 0.15 x 0.10 mm^3 with wavelength, λ=0.71073 Å. 25 well-centered reflections in the range $1.61<\theta<26.66$ and least square refinement was done to determine Lattice parameters. SAINT software was used for the integration of data. For the data collection, indexing reflections and the unit cell parameters Bruker SMART software was used. By direct methods procedure using SHELXS97 software, the structure of the title compound was solved and refined by F^2 full matrix least squares technique. 1218 reflections were found unique out of 3241 recorded reflections. Absorption corrections were not applied.

The positions of the hydrogen atoms were geometrically fixed for the title compound and these were allowed to ride on the corresponding non-hydrogen atoms. Using different Fourier maps the hydrogen attached to hetero atoms were located and refined with isotropic displacement co-efficient. The final refinement cycle converged R = 3.2% and $wR(F^2)$ = 7.9%. Atomic scattering factors were taken from International Tables of X-ray crystallography. The ORTEP view and packing of the molecule (title compound) are shown in Figure No.3 and Figure No.4, respectively.

Screening of antibacterial and antifungal activity

Purified compound (1,3 Benzoxazol-2(3H)-one) and varying solvent polarity of the flower extracts were tested against the above mentioned microorganisms. It is inferred from the results that the methanolic extract showed good inhibition than other solvent extracts. Purified compound also have proven to inhibit the growth of microorganisms in a comparable range with that of the standard drug.

Supplementary data

The crystal data, refinement parameters and details of structure analysis are summarized in Table No.1. Atomic coordinates, relevant geometrical parameters (bond length and bond angles), thermal coordinates, hydrogen coordinates and torsion angles are shown in Table No.2-6, respectively. The Zone of Inhibition values for the antimicrobial activity is given in Table No.7 and Figure No.5a and 5b.

Table No.1: Crystal data and refinement

S.No	Parameters	Values and Formula
1	Empirical formula	C7 H5 N O2
2	Formula weight	135.12
3	Temperature	293(2) K
4	Wavelength	0.71073 Å
5	Crystal system	Orthorhombic
6	Space group	P 2_1 2_1 2_1
7	Unit cell dimensions	a = 4.444 Å, b = 6.650 Å, c = 20.992 Å
8	Volume	620.4(9) A^3
9	Z, Calculated density	4, 1.447 Mg/m^3
10	Absorption coefficient	0.108 mm^{-1}
11	F(000)	280
12	Crystal size	0.20 x 0.15 x 0.10 mm
13	Theta range for data collection	1.94 to 27.95° deg
14	Limiting indices	-5<=h<=5, -6<=k<=8, -27<=l<=19
15	Reflections collected / unique	3241 / 1218 [R(int) = 0.0234]
16	Completeness to theta	27.95 88.9 %
17	Absorption correction	None
18	Max. and min. transmission	0.9892 and 0.9786
19	Refinement method	Full-matrix least-squares on F^2
20	Data / restraints / parameters	1218 / 0 / 91
21	Goodness-of-fit on F^2	1.072
22	Final R indices [I>2sigma(I)]	R1 = 0.0318, wR2 = 0.0785
23	R indices (all data)	R1 = 0.0354, wR2 = 0.0815
24	Absolute structure parameter	-0.4(14)
25	Largest diff. peak and hole	0.138 and -0.214 $e.A^{-3}$

Table No.2: Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3). U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor

S.No	Atomic group	x	y	z	U(eq)
1	C(1)	5518(3)	-101(2)	3490(1)	44(1)
2	C(2)	4220(3)	-1977(2)	3550(1)	59(1)
3	C(3)	2279(4)	-2248(3)	4055(1)	68(1)
4	C(4)	1662(4)	-733(3)	4483(1)	68(1)
5	C(5)	2968(4)	1144(3)	4424(1)	58(1)
6	C(6)	4887(3)	1394(2)	3921(1)	44(1)
7	C(7)	8147(4)	2648(2)	3231(1)	55(1)
8	N(1)	7542(3)	732(2)	3063(1)	54(1)
9	O(1)	9808(3)	3860(2)	2989(1)	82(1)
10	O(2)	6490(2)	3100(1)	3762(1)	54(1)

Table No.3: Bond lengths [Å] and angles [°]

S.No	Bond lengths [Å]	Angles [°]
1	C(1)-C(6)	1.3727(19)
2	C(1)-C(2)	1.380(2)
3	C(1)-N(1)	1.3852(17)
4	C(2)-C(3)	1.379(3)
5	C(2)-H(2)	0.9300
6	C(3)-C(4)	1.377(3)
7	C(3)-H(3)	0.9300
8	C(4)-C(5)	1.382(3)
9	C(4)-H(4)	0.9300
10	C(5)-C(6)	1.368(2)
11	C(5)-H(5)	0.9300
12	C(6)-O(2)	1.3806(17)
13	C(7)-O(1)	1.2057(19)
14	C(7)-N(1)	1.349(2)
15	C(7)-O(2)	1.3692(18)
16	N(1)-H(1)	0.8600

Table No.4: Anisotropic displacement parameters (A^2 x 10^3). The anisotropic displacement factor exponent takes the form: $-2\pi^2$ [h^2 a^{*2} U_{11} + ... + 2 h k a* b* U_{12}]

C(6)-C(1)-C(2)	120.65(13)
C(6)-C(1)-N(1)	105.58(12)
C(2)-C(1)-N(1)	133.76(13)
C(3)-C(2)-C(1)	116.70(15)
C(3)-C(2)-H(2)	121.7
C(1)-C(2)-H(2)	121.7
C(4)-C(3)-C(2)	122.05(16)
C(4)-C(3)-H(3)	119.0
C(2)-C(3)-H(3)	119.0
C(3)-C(4)-C(5)	121.24(15)
C(3)-C(4)-H(4)	119.4
C(5)-C(4)-H(4)	119.4
C(6)-C(5)-C(4)	116.15(14)
C(6)-C(5)-H(5)	121.9
C(4)-C(5)-H(5)	121.9
C(5)-C(6)-C(1)	123.21(13)
C(5)-C(6)-O(2)	127.46(12)
C(1)-C(6)-O(2)	109.33(12)
O(1)-C(7)-N(1)	130.04(15)
O(1)-C(7)-O(2)	121.75(16)
N(1)-C(7)-O(2)	108.21(12)
C(7)-N(1)-C(1)	109.79(12)
C(7)-N(1)-H(1)	125.1
C(1)-N(1)-H(1)	125.1
C(7)-O(2)-C(6)	107.07(11)

	x	y	z	U(eq)
H(2)	4634	-3007	3263	70
H(3)	1359	-3492	4109	82
H(4)	341	-978	4818	82
H(5)	2565	2177	4710	70
H(1)	8299	120	2740	65

Table No.5: Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3)

	U11	U22	U33	U23	U13	U12
C(1)	43(1)	45(1)	44(1)	2(1)	-5(1)	5(1)
C(2)	65(1)	44(1)	67(1)	-2(1)	-14(1)	6(1)
C(3)	61(1)	60(1)	84(1)	22(1)	-6(1)	-12(1)
C(4)	52(1)	87(1)	64(1)	21(1)	2(1)	-8(1)
C(5)	53(1)	71(1)	51(1)	-5(1)	5(1)	2(1)
C(6)	44(1)	44(1)	45(1)	1(1)	-3(1)	2(1)
C(7)	61(1)	59(1)	46(1)	7(1)	2(1)	-3(1)
N(1)	61(1)	58(1)	42(1)	-4(1)	7(1)	6(1)
O(1)	95(1)	83(1)	69(1)	17(1)	15(1)	-27(1)
O(2)	64(1)	46(1)	54(1)	-4(1)	7(1)	-6(1)

Table No.6: Torsion angles [°]

C(6)-C(1)-C(2)-C(3)	0.4(2)
N(1)-C(1)-C(2)-C(3)	179.34(14)
C(1)-C(2)-C(3)-C(4)	-0.2(2)
C(2)-C(3)-C(4)-C(5)	0.1(2)
C(3)-C(4)-C(5)-C(6)	0.0(2)
C(4)-C(5)-C(6)-C(1)	0.2(2)
C(4)-C(5)-C(6)-O(2)	-179.22(13)
C(2)-C(1)-C(6)-C(5)	-0.3(2)
N(1)-C(1)-C(6)-C(5)	-179.57(13)
C(2)-C(1)-C(6)-O(2)	179.14(11)
N(1)-C(1)-C(6)-O(2)	-0.09(14)
O(1)-C(7)-N(1)-C(1)	178.95(16)
O(2)-C(7)-N(1)-C(1)	-1.03(15)
C(6)-C(1)-N(1)-C(7)	0.69(15)
C(2)-C(1)-N(1)-C(7)	-178.40(15)
O(1)-C(7)-O(2)-C(6)	-179.04(14)
N(1)-C(7)-O(2)-C(6)	0.94(15)
C(5)-C(6)-O(2)-C(7)	178.93(14)
C(1)-C(6)-O(2)-C(7)	-0.52(14)

Table No.7: Inhibition zone values for antimicrobial activity

Compound and solvent extracts	Concentration (µl)	Zone of Inhibition (mm)					
		sa	**mt**	**ec**	**pa**	**Ca**	**an**
Purified compound	25	5	9	4	4	4	4
	50	7	10	4	11	4	4
	75	13	13	11	17	10	8
	100	**21**	**20**	**12**	**23**	**20**	**15**
	AD	35	34	28	25	22	20
MET	25	8	12	11	16	9	8
	50	7	14	15	18	11	11
	75	10	20	16	23	11	11
	100	**23**	**24**	**25**	**23**	**25**	**19**
	AD	32	10	30	28	20	20
EA	25	2	12	10	11	9	8
	50	15	15	15	16	9	8
	75	15	15	16	18	11	11
	100	**22**	**22**	**23**	**23**	**16**	**23**
	AD	35	28	24	27	25	25
CF	25	4	10	4	8	4	12
	50	4	12	8	9	6	13
	75	5	14	11	9	11	18
	100	**18**	**15**	**21**	**20**	**22**	**22**
	AD	39	30	42	32	31	24
PET	25	9	8	9	9	8	9
	50	9	8	9	11	9	10
	75	11	9	11	11	12	10
	100	**11**	**11**	**11**	**12**	**12**	**12**
	AD	33	19	30	31	24	25
Aq	25	10	8	10	9	10	9
	50	12	11	12	9	12	10
	75	12	17	12	9	12	10
	100	**20**	**20**	**20**	**12**	**18**	**18**
	AD	35	15	32	27	20	22

sa- *Staphylococcus aureus;* mt-*Mycobacterium Tuberclosis;* ec-*Escherichia coli;* pa- *Pseudomonas aeruginosa;* ca- *Candida albicans;* an-*Aspergillus niger;* AD- Antibiotic Disc[Ciprofloxacin (2 µg)]; Purified compound – 1,3 Benzoxazol-2(3H)-one; MET- methanol; EA- Ethyl acetate; CF- Chloroform; PET- Petroleum ether; Aq- Aqueous.

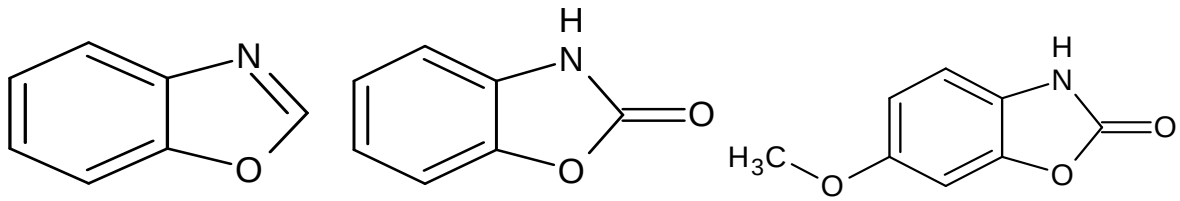

Figure No.1: The chemical diagram: (a) Benzoxazole, (b) 1,3-Benzoxazol-2(3H)-one and (c) 6-Methoxy-2(3H)-BOA

Figure No.1 (a): *Crossandra infundibuliformis* plant

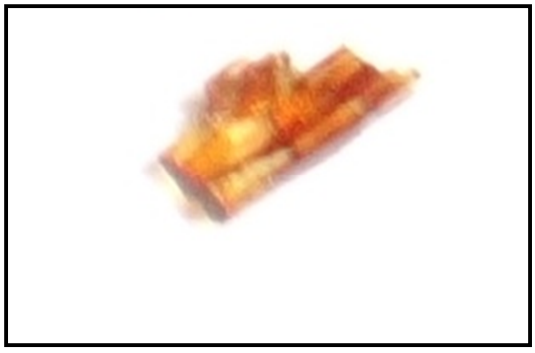

Figure No.1 (b): 1, 3-Benzoxazol-2(3H)-one Crystal

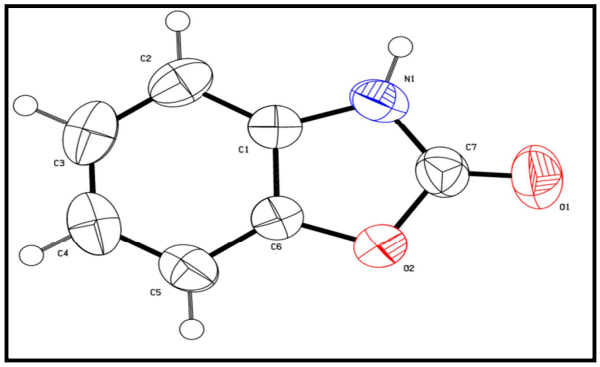

Figure No.3: ORTEP view of 1,3-Benzoxazol-2(3H)-one crystal structure

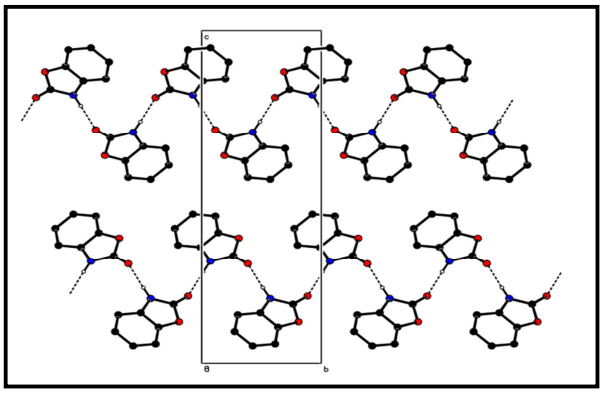

Figure No.4: Packing of molecules in the unit cell down 'a' axis

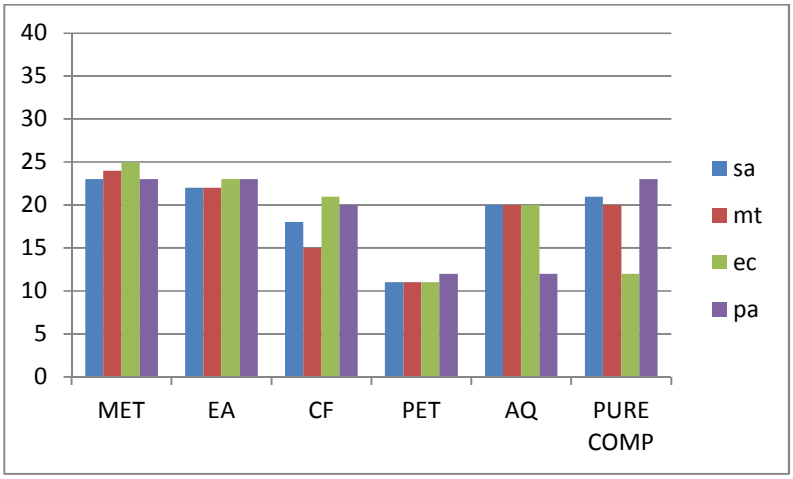

Figure 5: a) Antibacterial activity with maximum concentration (100 µl)

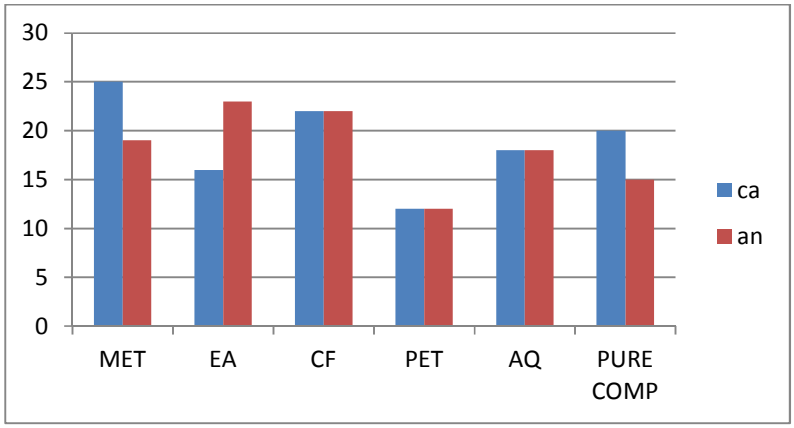

Figure 5: b) Antifungal activity with maximum concentration (100 µl)

CONCLUSION

Crossandra infundibuliformis is an ornamental plant reported to have medicinal properties. Many research works is carried out on the leaves of this plant. Hence, we focused our study on the purification of a compound from the flower part of this plant and also to screen the antimicrobial activity. In this study, we have purified a compound (1,3 Benzoxazol-2(3H)-one) from the flower extract of *Crossandra infundibuliformis* for the first time. Antimicrobial screening of the purified compound and varying polarity solvent extracts of this flower against the bacterial and fungal strains have showed comparable results with that of the standard drug used. Higher the concentration of the extracts (test sample), the inhibition level is good. Alike, the leaf extracts of this plant, flower extracts and also the purified compound have proven to have antimicrobial activity. In future, other medicinal properties could be studied in the flower part of this plant.

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CONFLICT OF INTEREST

None declared.

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