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A Preliminary Screening of Analgesic, Cytotoxic and Antidiarrhoeal Activities of *Ardisia solanacea* (Poir) Roxb.

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Abstract: The current research was performed to study the analgesic, cytotoxic and antidiarrhoeal activities of ethanolic extract of the stem and root of *Ardisia solanacea* (Poir) Roxb. The extracts showed significant analgesic activity in acetic acid induced writhing inhibition in mice. The extract of stem and root showed writhing inhibition of 67.05% and 71.05% respectively at the dose of 500mg/kg. In the brine shrimp lethality bioassay, the extract showed minor cytotoxicity in stem and root with LC_{50} of 165.799 and 185.485 μ g/ml respectively whereas, the standard drug vincristine sulfate showed LC_{50} of brine shrimp nauplii at 0.482 μ g/ml. Antidiarrhoeal activity was investigated on animal model, Swiss Albino mice. Ethanolic extracts of stem and root of *Ardisia solanacea* (Poir) Roxb. were effective in reducing faeces in castor oil induced diarrhoeal mice as 47.2% and 53.1% respectively at a dose of 500mg/kg. The above results indicated that the ethanolic extract of the plant, *Ardisia solanacea* (Poir) Roxb. has significant analgesic activity with moderate antidiarrhoeal effect and has minor cytotoxic activity. Further investigations can be done to find out the active constituents responsible for these effects.

Keywords: Analgesic, Cytotoxicity, Antidiarrhoeal, Writhing Inhibition.

INTRODUCTION

The importance of medicinal plants among all the natural sources is inevitable (Majumder *et al.*, 2014). The phytochemicals obtained from medicinal plants provide the active molecules in the modern medicines (Raju *et al.*, 2013). According to World Health Organization (WHO), about 80% of the world population still uses medicinal plants for different medical purposes (Majumdar *et al.*, 2014). Around the world, almost 500 species of *Ardisia* are found throughout tropical and subtropical regions. Several of those species have been used as ornamental plants, medicines and foods (Kobayashi *et al.*, 2005). *Ardisia solanacea* (Poir) Roxb. (Family: Myrsinaceae), Bengali/Vernacular Name: Ban Jam is a small shrub. Leaves are large, 16 cm long, flowers pink. The plant possesses stimulant and carminative properties. Roots are febrifuge and used in diarrhoea and rheumatism. Root-bark boiled water is used to wash sores and paste of root-bark is applied to heal sores. (Ghani *et al.*, 2003). The plant is used in the treatment of diarrhea, gout, dysmenorrhoea, mental disorder, rheumatic arthritis, skin sore and vertigo (Khatun *et al.*, 2013). The other species of the *Ardisia* also showed cytotoxic, thrombolytic and antioxidant properties. Among various chemical compounds, roots and stems contain bauerenol and leaves contain bauerenol, α - & β - amyrin (Asolkar *et al.*, 1992). The leaves also contain hydrocarbons, fatty acid, triterpenoid alcohols and sterols. Bergenin is an important constituent of the plant.

(Ghani *et al.*, 2003). In the present work, an attempt was made to justify the traditional uses as per scientific experiments. Upon enough literature survey it is found that a very few work has been performed to evaluate the rationale use of this plant in traditional medicine of Bangladesh. In this research, we therefore, aim to evaluate the analgesic, antidiarrhoeal, cytotoxic activities of the ethanolic extract of stem and root of *A. Solanacea* (Poir) Roxb.

Materials and Methods

Sample collection and extraction

For this present investigation, the plant *A. Solanacea* (Family: Myrsinaceae) was collected from horticulture field of Khulna University, Khulna. The plants were carefully cleaned and separated from undesired materials. After complete drying, the leaves were removed from stem. The stem and roots were chopped into small pieces and dried. Those fragmented pieces were covered with a clean cloth to protect from dust and were allowed to dry under sunshade. After complete drying, the stems and roots were ground into coarse powder using suitable electrical grinder.

Powders of stem and root were extracted by Soxhlet extraction method. For this, a tumble was made by Whatman filter paper and it was filled with powder and was put in the Soxhlet chamber. The height of powder in the tumble was below the level of Syphon tube of the Soxhlet chamber. 98% ethanol was placed in the spherical flask which is attached below the Soxhlet chamber. A condenser which was attached at the top of the Soxhlet chamber was provided with cold water. The spherical flask, thus the entire system was heated by putting it on an electric heater. The ethanolic extract was collected from the spherical flask and was taken into the flask of Rotary Evaporator to evaporate the ethanol to get the extracts (yield value: 30% for stem and 24% for root).

Animals

For the experiment, Swiss-Albino mice of both sexes (body weight: 20-25 gm) were obtained from research branch of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The animals were kept in the standard polypropylene cage and maintained in the animal house of Pharmacy Discipline, Khulna University, Khulna for the period of 10 days prior to the experiment. Standard diets (formulated by ICDDR, B) were provided to the animals under standard laboratory conditions (room temperature: 25±2° C, relative humidity: 60-65% and 12 hours light-dark cycle).

Drugs (as standard)

Diclofenac sodium, Lopramide, Vinchristine sulphate (Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh)

Preliminary Phytochemical screening

The stem and root extracts were subjected to standard tests to detect the presence of major phytochemical groups (Harborne, 1998; Kokate, 1994).

Analgesic activity test

- **Acetic acid induced writhing method**

This test was performed following the method developed by B.A. Whittle (1964) (Whittle *et al.*, 1964). The writhing induced by acetic acid liberates endogenous substances that excite the pain nerve ending and causes algesia (Ahmed *et al.*, 2004). The randomly selected experimental animals were divided into six groups (each group contains 5 mice) named as control group, positive control, test group 1, 2, 3 and 4. Control mice were treated with tween-80 (10% solution 1ml/kg) and positive control with diclofenac sodium at the dose of 25 mg/kg i.p. Test group 1 and 2 were given 250 and 500 mg/kg of stem extract i.p. respectively whereas test group 3 and 4 were treated with 250 and 500 mg/kg of root extract i.p. respectively. After 1hr of the administration of the extract and standard drug, the writhing was induced in mice by injecting 0.7% acetic acid solution 0.1ml/10g body weight i.p. The writhing was counted for 15 minutes after 5 minutes of acetic acid administration.

Determination of Cytotoxic Activity

Cytotoxicity of *Ardisia solanaceae* was investigated by brine shrimp (*Artemia salina*) lethality bioassay (Meyer *et al.*, 1982). Small Amount (5-6g) of brine shrimp egg was hatched in saline water for 48hr to produce nauplii. The saline water was prepared by dissolving 30mg pure NaCl and 53mg table salt into 1.5 litre water. 32 mg extract of both stem and bark of *Ardisia solanaceae* (Poir) Roxb. was taken in a 10 ml volumetric flask separately, few drops of tween-20 (10% solution) was added and the volume was adjusted to 10 ml by sea water. From this, nine different concentrations (320, 160, 80, 40, 20, 10, 5, 2.5 and 1.25 µg/ml) were prepared for both stem and root extract in nine test tubes. For standard solution vincristine sulphate was prepared in different concentrations (5, 2.5, 1.25, 0.625, and 0.313µg/ml). In control group only saline water was used. 10 nauplii were placed in each test tube and the volume was adjusted to 5ml by saline water. After 24 hr the live brine shrimp were counted and recorded. The test was performed thrice and the LC₅₀ (lethal concentration) was determined by ldp line software.

Determination of Anti Diarrhoeal Activity

The stem and root extract of *A. solanaceae* were subjected to antidiarrhoeal activity test by castor oil induced diarrhoea in mice (Shilpi *et al.*, 2006). The animals were grouped into six groups as control, positive control test group 1,2,3 and 4 (5 animals in each group). The control group received only distilled water containing 1% Tween-80, the positive control received Loperamide (3mg/kg-body weight), the test group 1 and 2 were given stem extract at a dose of 250 and 500mg/kg respectively and the test group 3 and 4 were given root extract at a dose of 250 and 500mg/kg respectively. After 30 minutes of this work the mice were administered castor oil (0.3ml to each) and placed in separate case individually. The floor of the case was lined with adsorbent paper and the paper was changed per hour of total 4hours observation period. The latent period was observed for each mouse and the total number of faecal output including diarrhetic faeces was recorded. A numerical score based on stool consistency was counted as follows: normal stool=1 and watery stool=2.

Table 1: Effect of Stem and Root of *Ardisia solanaceae* on Acetic Acid Induced Writhing in Mice.

Animal Group(n=5)	Treatment	Writhing Count (%)	% Inhibition
Control	1% tween-80 solution in water	17.6 ± 0.93 (100)	0
Positive Control	Diclofenac sodium (25mg/kg)	5.2 ± 0.62* (29.54)	76.14
Test group 1	Stem Extract (250mg/kg)	9.6 ± 0.68* (54.55)	45.45
Test group 2	Stem Extract (500mg/kg)	5.8 ± 1.62* (32.95)	67.05
Test group 3	Root Extract (250mg/kg)	8.5 ± 1.2* (44.73)	55.26
Test group 4	Root Extract (500mg/kg)	5.1 ± 0.96* (26.84)	71.05

Values are expressed as mean ± SEM, n=No. of mice *: P < 0.001; Vs Control

Table 2: Result of Brine Shrimp Lethality Bioassay of Ethanolic Extracts of *Ardisia solanaceae* (Stem and Root) Against Standard Vincristine Sulphate.

Conc. of standard (µg/ml)	Conc. of Extract of Stem and Root (µg/ml)	Number of Shrimp (in each test tube)	Number of Alive Shrimp in Control	Number of Alive Shrimp in standard	Number of Alive Shrimp in stem extract	Number of Alive Shrimp in root extract	% Mortality (Standard)	% Mortality (Stem)	% Mortality (Root)
0.312	5	10	10	5.66±0.27	10	10	43.33±2.72*	00	00
0.625	10	10	10	4.66±0.27	9.33±0.27	8.66±0.27	53.33±2.72*	6.66±2.72*	13.33±2.72*
1.25	20	10	9	3.33±0.27	8.33±0.27	8.66±0.272	66.66±2.72*	16.66±2.72*	13.33±2.72*

2.5	40	10	9	1.66±0.27	7	7.33±0.27	83.33±2.72*	30*	26.66±2.72*
5	80	10	10	0.66±0.27	5.33±0.27	6.33±0.27	93.33±2.72*	46.66±2.72*	36.66±2.72*
10	160	10	10	00	5.33±0.27	5.33±0.27	100*	46.66±2.72*	46.66±2.72*
20	320	10	10	00	4.33±0.27	4±0.47	100*	56.66±2.72*	60±4.71*

Values are expressed as mean±SEM, SEM=Standard error of Mean, %=Percentage, *P<0.001 vs Control

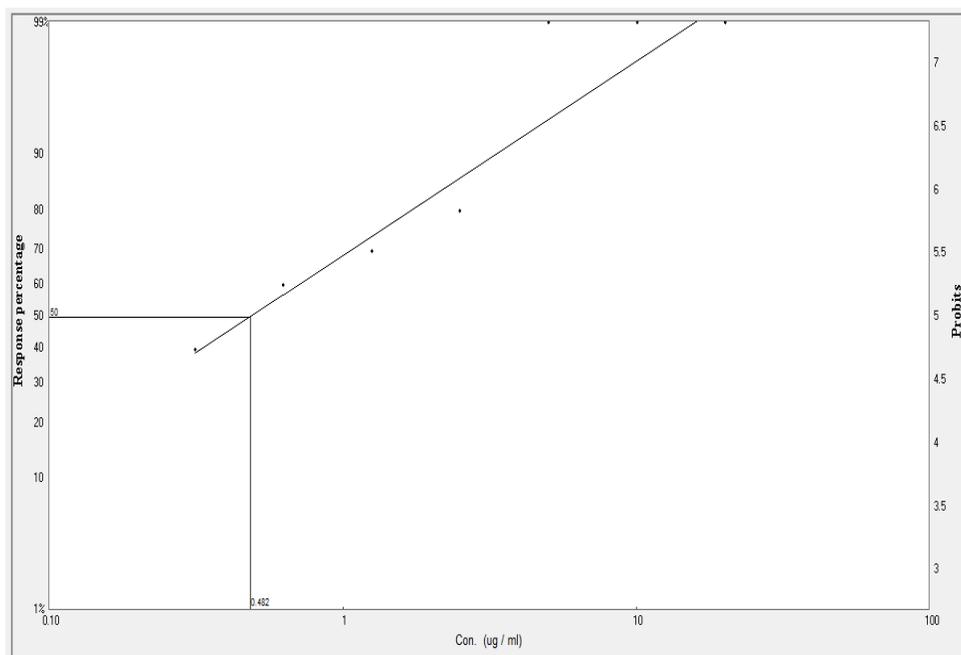


Figure 1: LC₅₀ determination of standard vincristin sulphate

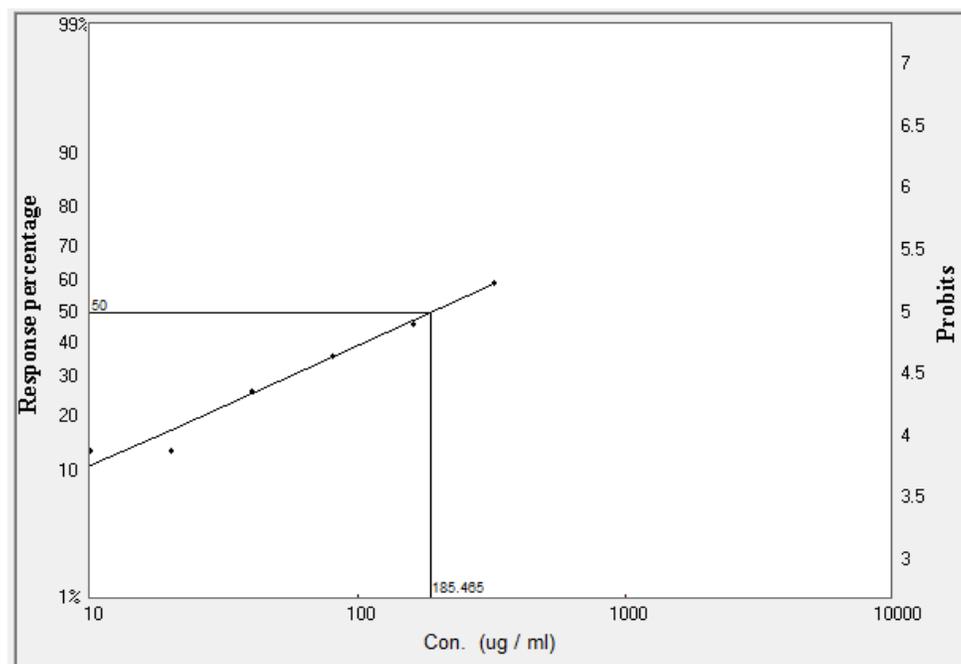


Figure 2: LC₅₀ determination of root

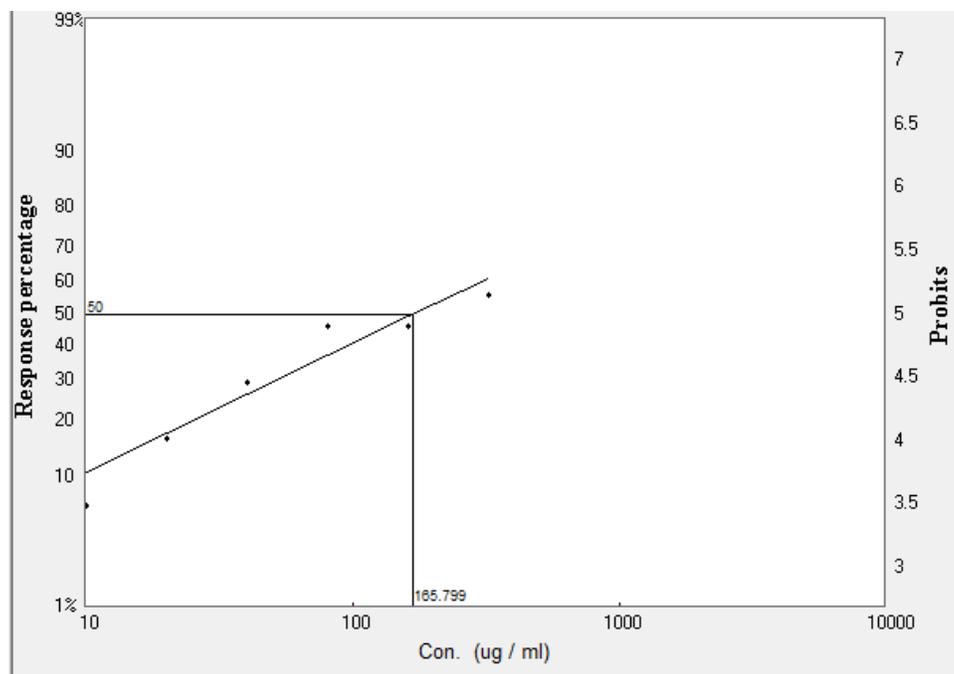


Figure 3: LC₅₀ determination of stem

Table 3: Effect of *A. Solanaceae* stem and root extracts on castor oil-induced diarrhoea.

Treatment	Latent period (hr)	Total no. of faeces in 4 hours	Feaces reduction (%)
Control (Water containing 1% tween 80, 10ml/kg)	0.46±0.88	18±1.87	–
Positive control (Loperamide 3mg/kg)	2.45±0.73	5±0.84**	72.2
Test group-1 (Et. Extract of stem 250mg/kg)	0.90±1.34	14.2±1.13**	21.1
Test group-2 (Et. extract of stem 500mg/kg)	1.5±0.84	9.5±0.88**	47.2
Test group-3 (Et. Extract of root 250mg/kg)	1.1±1.87	13.3±1.55**	26.1
Test group-4 (Et. extract of root 500mg/kg)	1.9±1.13	8.4±1.34**	53.3

Values are expressed as mean±SEM, SEM=Standard error of Mean, %=Percentage, **P<0.001 vs Control

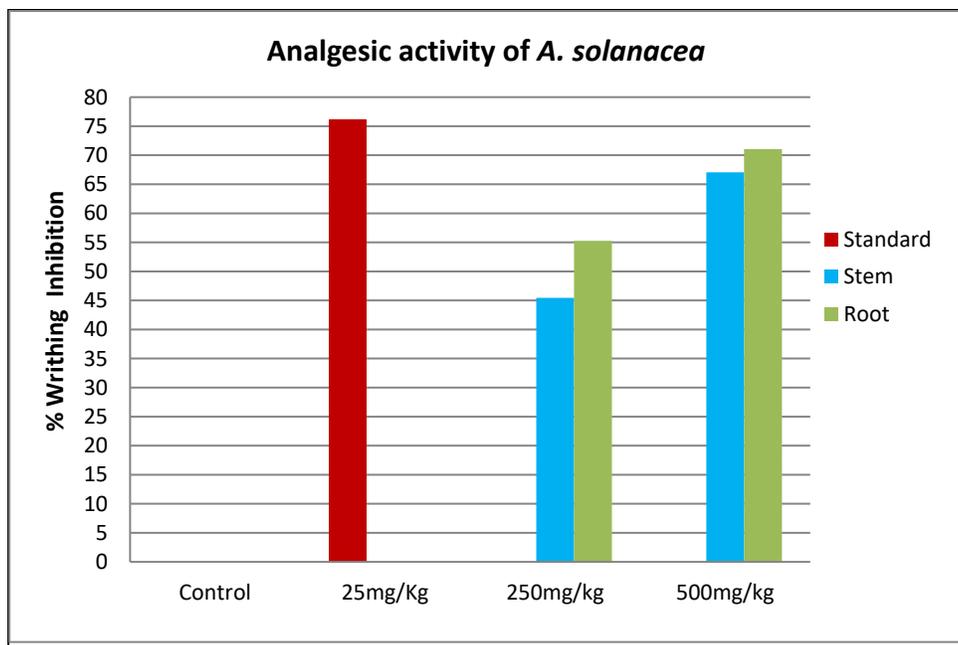


Figure 4: Analgesic activity of ethanolic extract of stem and root of *A. solanacea*

Result

Preliminary Phytochemical screening

The preliminary phytochemical analysis of *A. solanacea* indicated the presence of alkaloid, saponin, phytosterol, tannin, flavonoid, protein and amino acid and absence of glycoside and terpene in both stem and root.

Analgesic activity test

In the acetic acid induced writhing test, the stem and root extract showed significant analgesic activity in compared to that of the standard diclofenac sodium at a dose of 500mg/kg by inhibiting the writhing in mice. (Table 1)

Determination of Cytotoxic Activity

In brine shrimp lethality bioassay, the stem and root extracts were found to show lethal activity against brine shrimp nauplii and LC_{50} was found at 165.799 and 185.485 μ g/ml respectively and the LC_{50} for standard vincristine sulphate was 0.482 μ g/ml. (Fig 1, 2 & 3)

Determination of Anti Diarrhoeal Activity

In the castor oil induced diarrhoea on mice, the root extracts reduced faeces more than that of the stem extract in both the doses. The results obtained were comparable to that of the standard drug. (Table 3)

Discussion

The ethanolic extract of stem and root of *Ardisia solanacea* were investigated for analgesic, cytotoxic and antidiarrhoeal activity. Results showed that the plant parts posse's significant analgesic, cytotoxic and moderate antidiarrhoeal activities.

A. solanaceae is traditionally used in the treatment of gout and so the stem and root extract were investigated for analgesic activity by acetic acid induced writhing method. In intraperitoneal administration of acetic acid various endogenous substances like histamine, serotonin, prostaglandin, bradykinin and substance P are released and excite the pain nerve endings (Taesotikul *et al.*, 2003). Among the prostanoids, PGE2 and PGF2 α

in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Deradt *et al.*, 1980). So in the observed activity of *A. Solanaceae*, stem and root might contain pharmacologically active constituents that can block the release of endogenous substances or the effects responsible for the algisia.

The brine shrimp lethality bioassay has been used extensively in the primary screening of the crude extracts as well as the isolated compounds to evaluate the cytotoxicity, which could also provide an indication of possible cytotoxic properties of the test materials (Ali *et al.*, 2001). This cytotoxic bioassay has also a good correlation with the killing of human tumour cell lines. The inhibitory effect of the extracts may be due to the toxic compounds present in the active fraction that possess ovicidal and larvicidal properties. The metabolites either affected the embryonic development or slay the eggs. Therefore the cytotoxic effects of the plant extracts enunciated that it can be selected for further cell line assay because there is a correlation between cytotoxicity and activity against the brine shrimp nauplii using extracts (Manila *et al.*, 2009).

In our present study the cytotoxic assay was performed using brine shrimp lethality bioassay. Both the extracts of stem and root showed minor cytotoxic activity as compared to that of standard (Table 3).

The traditional use of the plant *A. solanaceae* in diarrhoea encouraged to carry out the antidiarrhoeal activity test. Castor oil was used to produce diarrhoea in the mice as it produces ricinoleic acid by the action of lipase in the small intestine (Robert *et al.*, 1976) that mediates its action by releasing endogenous prostaglandins (Tanaru *et al.*, 2012) and facilitates the accumulation of fluid in the intestine by inhibiting absorption and enhancing secretion of fluid and electrolytes (Racusen *et al.*, 1979). Moreover, this metabolite also stimulates the peristaltic movement of GI smooth muscles (Matias *et al.*, 1978). In this test the result showed that the stem and root extract of *A. solanaceae* significantly ($P < 0.001$) reduced the diarrhoeal event of mice and supported its traditional use. The antidiarrhoeal action may also be associated with the inhibition of prostaglandin production and this matter is validated by the fact that castor oil induced diarrhea is related to stimulation of the prostaglandins synthesis (Robert *et al.*, 1976; Taufiq-Ur-Rahman *et al.*, 2005).

Conclusion

The present study expressed that the ethanolic extract of stem and root of *A. Solanacea* posses significant analgesic activity with moderate antidiarrhoeal activity and minor cytotoxic activities. Result of analgesic potentiality test justifies the traditional uses of this plant in dysmenorrhea, gout and rheumatic arthritis but the result of antidiarrhoeal activity does not satisfy its traditional use in diarrhoea. As the plant showed a little activity in brine shrimp lethality bioassay, it can be a safely used as drug without cytotoxicity. Since the extracts are not reported about the phytochemical compounds present in it, it is difficult to attribute these observed activities to any chemical compounds responsible for these. Hence, to better understand the mechanism of such actions of *A. solanacea*. Further studies are suggested to find the exact compound (s) responsible for its observed activity.

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