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EVALUATION OF PHARMACOGNOSTICAL, PHYTOCHEMICAL, PRELIMINARY, ANTHELMINTIC AND ANTI-OXIDANT POTENTIAL OF LEAF OF *EUPHORBIA HIRTA* LINN

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Keywords:

Euphorbia hirta, Pharmacognostical study, Anthelmintic activity, Antioxidant activity

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ABSTRACT: To evaluate the Pharmacognostic properties, phytochemical screening, anthelmintic and anti-oxidant potential of the leaves of *Euphorbia Hirta Linn*. Microscopic and macroscopic characteristics of fresh and dried leaf samples were analysed. serial exhaustive extraction was done with various of solvents: Aqueous, Methanol, using Soxhlet apparatus. Physicochemical parameters such as moisture content, extractive values, ash content of leaf powder were also determined. The results revealed that the leaves extracts contain carbohydrates, alkaloids, steroids, Saponins, terpenoid, flavonoids etc., The results of anthelmintic study indicated that the methanolic and aqueous extracts significantly exhibited paralysis ($P < 0.01$) in worms in lower doses (5, 10 and 15 mg/ml) and also caused death of worms especially at higher concentration of 15mg/ml, as compared to standard drug. These studies may be helpful for identification of *Euphorbia Hirta Linn plant*. This phytochemical may be responsible for the therapeutic activity of the plant. Further it useful for researcher.

INTRODUCTION: It is commonly known as asthma plant, asthuma weed, dudhy.

Botanical Description: *Euphorbia hirta* Linn is the plant of euphorbiaceae family. It is commonly known as Asthma plant, Dove milk, Garden spurge, red euphorbia, milk weed plant. It is distributed throughout the temperate or tropical parts of India, Asia, Australia, and Africa, often found in lowland, paddy fields, gardens, waste places, and in the roadsides. It is native to central America¹. *Euphorbia hirta* is a small annual, erect or ascending, branched prostrate herb with branches reaching 60 cm in height, reddish or purple, with abundant latex and is hairy.

Leaves are opposite, elliptical; its base is very dissimilar or unequal; one side is cuneate, the other side is obliquely rounded; the apex is acute, length is 3-4 cm and width is 1-1.4 cm, they are darker on the upper surface. The flowers are unisexual; the male flowers are sessile, the bracteoles are linear, fringed, the perianth is absent, and possesses 1-stamen, the female flowers have small pedicel, the perianth is rimmed, the ovary is superior, covered with minute hairs, 3-celled, possesses 3-styles, small, and the apex is 2-fold. Flowering duration is usually throughout the year. Fruits are yellow, hairy, three celled, 1-2mm in diameter. The plant grows in well-drained, slightly acidic, sandy, or gravelly soils. It usually grows in moist or dry habit in waste lands or as weed^{2,3}.

Taxonomical Classification^{4,5}:

Kingdom: Plantae

Sub kingdom: Viridiplantae

Infra Kingdom: Streptophyta

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Division: Tracheophytes

Sub Division: Spermatophyte

Infra Division: Angiosperms

Class: Magnoliopsida

Super Order: Rosanae

Order: Malpighiales

Genus: Euphorbia

Family: Euphorbiaceae

Species: Euphorbia hirta

Authority: Linn

Chemical Constituents⁶⁻¹⁰: From the literature survey, Phytochemical analysis of leaf extract showed the presence of carbohydrates, reducing sugars, terpenoids, alkaloids, steroids, tannins, proteins, fats, oils, mucilages, glycoside, saponin, coumarin, anthroquinones, chlorophyll, and carotenoids. Flavonoids compounds like quercetin, quercitrin, quercitol and its derivatives such as rhamnose, quercetin rhamnoside, chlorophenolic acid, rutin, leucocyanidin, myricitrin, cyaniding 3,5- diglucoside, camphol, flavonol, inositol, tetraerol, β -sitosterol, and kaempferol are found in *Euphorbia hirta*. Afzelin, euphorbin-A, euphorbin-B, euphorbin-C, euphorbin-D, gallic acid, and protocatechuic acid were also isolated from the aerial parts of the plants. D-phytosterols such as campesterol, cholesterol and stigmasterol are also present. Tannin categories of compound such as dimeric hydrolysable dehydro ellagic tannins, and terchebin, the monomeric hydrolysable tannins geranin and benzyl gallate etc are found in this plant. Acids categories of compound like Ellagic, gallic, tannic, maleic and tartaric acids are present in this plant.

Medicinal uses¹¹⁻¹⁴:

- The whole plant is used as astringent and haemostatic (stop bleeding); as poultice applied to ulcer, inflamed glands, oedema and also used in affection of childhood, cough and in worms.
- The plant is used to treat /cure severe pain such as headache, toothache, colic rheumatism, and pains occur during pregnancy.

- The extracted juice of this plant has emollient/sedative effect on the mucous layer of the respiratory and urinary tract.
- This plant is used to treat poison condition (antidote) and pain relief of scorpion stings and snakebites.
- The plant has known for increasing milk flow in women, and because of its milky latex, it is also used for different female disorders.
- The cold extract of the leaves is used to bath small babies with skin infections.
- It is used as anti-inflammatory, anti-microbial, anti-diarrheal, sedative, analgesic, anti-pyretic, anti-oxidant, anti-asthmatic, anti-tumour, larvicidal, diuretic, etc.
- It is extensively used traditionally to cure and prevent gastro-intestinal disorders, afflictions of mucous membranes, and respiratory system disorders.
- It also reported that the plant is used as anti-spasmodic, anti-diabetic, anti-inflammatory, anti-cancer curative agent.

MATERIALS AND METHODS:

Plant Collection and Authentication: The fresh leaves of the *E. hirta* were collected during the month of July-August from local area Banagahalli, Mandya, Karnataka, India. The plant was identified and authenticated by Dr. Thejesh Kumar, M. P, HOD Department of Botany Bharathi college, Bharathinagara and Mandya.

Worms Collection: Mature and healthy Indian earth worms *Pheretima posthuman* (Annelida) were collected from the VC-Farm, Mandya on 29/08/2024 to evaluate the anthelmintic property of the extracted drug. The collected earthworms were washed with normal saline or water to remove all faecal matter and were used for the anthelmintic study.

Plant Extraction: The plant material (leaves) was collected and washed with running tap water, chopped and shade dried. The dried plant will be pulverised with the help of an electric grinder.

Organoleptic Characteristics Studies: The powdered drug's taste, look, colour, and odour were all examined in the investigation of the organoleptic features. One milligram of powdered substance is placed between the thumb and forefingers to perform the odour test. The pungent ingredients were tested numerous times before their delayed release. The intensity of the smell was initially assessed by the following parameters: "No, Low, Sharp, and Strong" Then was determined odour type: "Aromatic, fruity". For taste, 5 grams of drugs are placed and kept in the mouth without swallowing, for 10 to 30 seconds. After spit sample, the mouth is rinsed and then enjoyed the taste: «Piquant, Fade, sour, bitter, sweet, salty, warm. This crucial study allows for drug identification and standardization. The appearance and color demanded observation.

Determination of Ash Value:

Determination of Total Ash: About 2g of powdered drug was weighed accurately and placed in tarred silica crucible and incinerated at 450°C in muffle furnace until free from carbon. Crucible cooled, kept in a desiccator and weighed. Same procedure was repeated to arrive at constant weight. The % of total ash obtained was calculated with reference to the air-dried drug.

Total ash value of powdered crude drug was recorded.

$$\text{Total ash value of sample} = 100 (Z - X) / Y$$

Z = Weight of total dish + ash (after complete incineration)

X = Weight of the empty dish

Y = Weight of the drug taken

Water Soluble Ash Value: Total ash was accurately weighed and boiled with 25ml of water for 5min, filtered on ash less filter paper. Insoluble matter was washed with hot water, ignited at near about 450°C temperature in muffle furnace. Cooled in a desiccators & weighed.

Determination of Extractive Value: Extractive value of crude drug determines the amount of active constituent extracted with solvents from a given amount of plant material.

Alcohol Soluble Extractive Value: About 5g of coarse powder of the crude drug was weighed and macerated in iodine flask with 100 ml of 70% v/v alcohol, for a duration of 24 hrs, with frequent shaking. Solution was filtered rapidly. Taking precaution against loss of alcohol, 25 ml of filtered solution was evaporated to dryness at 105°C in a tarred flat bottom Petri dish. The percentage of alcohol soluble extract was determined with reference to shade dried drug.

Water Soluble Extractive Value: About 5g of coarse powder of the crude drug was weighed and macerated in iodine flask with 100 ml of water. For a duration of 24 hrs, with frequent shaking. Solution was filtered rapidly; taking precaution against loss of water, 25 ml of filtered solution was evaporated to dryness at 105°C in a tarred flat bottomed Petri dish. The percentage of water-soluble extractive was determined with reference to the shade dried drug.

Phytochemical Screening: The freshly prepared different leaves extract was qualitatively tested for the presence of chemical constituents. They identified by characteristic colour changes and precipitation reactions using standard procedures.

Determination of Antioxidant Activity by Hydrogen Peroxide Scavenging Assay:

Chemical: Hydrogen peroxide, Phosphate buffer, Methanol.

Materials: Test tubes, volumetric flasks, Pippete, UV Spectrophotometer.

Standard: Ascorbic acid (20-100µg/ml)

Procedure:

- The extract (20, 40, 60, 80, 100 µg/ml)
- Standard (Ascorbic acid 20-100µg/ml) different concentration were dissolved in 3.4 ml of 0.1 M phosphate buffer (p^H 7.4) and mixed with 0.6ml of 40mM solution of hydrogen peroxide.
- Phosphate buffer used as blank.

Absorbance was measured at 230nm. The results are recorded

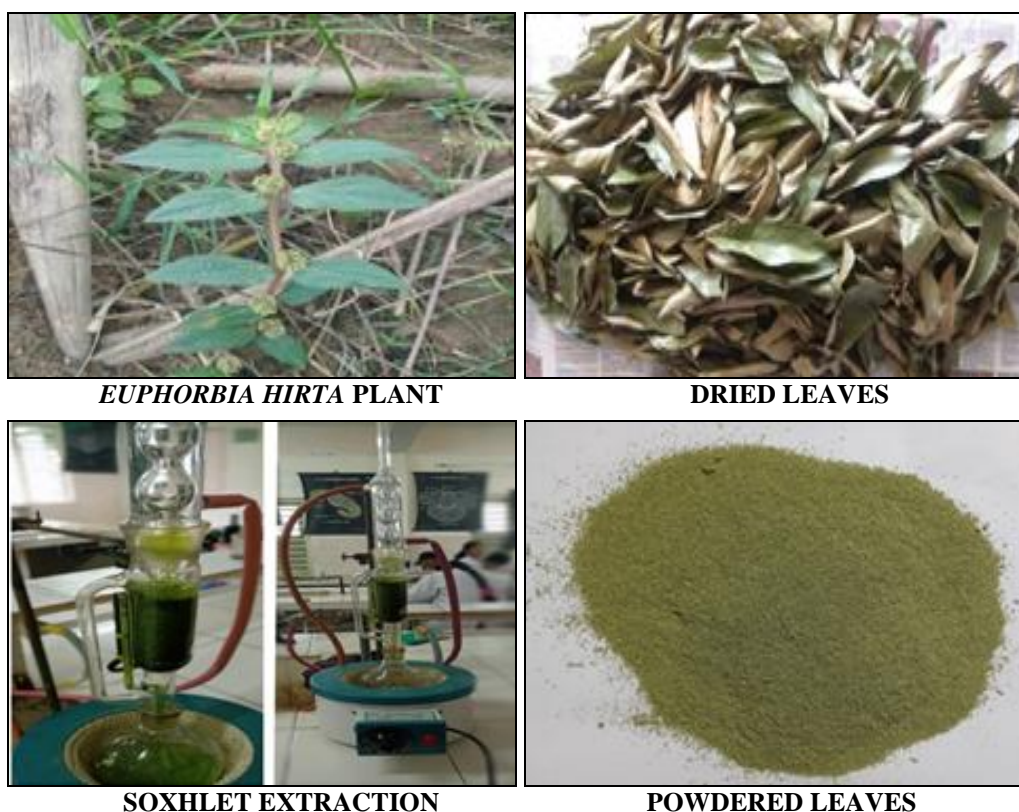
Anthelmintic Assay¹⁵⁻¹⁶: The anthelmintic assay was carried out as per the method of Ajayieoba et al with slight modifications. The assay was carried out on adult Indian earthworm *Pheretima posthuma*, due to its anatomical and physiological similarities with the intestinal roundworm parasites of human beings. *Pheretima posthuma* worms are easily available and used as a suitable model for screening of anthelmintic drug. The 50 ml formulations containing four different concentrations of each methanolic and aqueous extract (5, 10 and 15 mg/ml in distilled water) were prepared and six worms (same type) were placed in

it. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50 °C. Albendazole (5-15 mg/ml) was used as reference standard while distilled water as the control.

Drugs and Chemicals: Albendazole- as reference standard.

Chemicals: Methanol were used during the experimental protocol.

RESULT AND DISCUSSION:



EUPHORBIA HIRTA PLANT

DRIED LEAVES

SOXHLET EXTRACTION

POWDERED LEAVES

FIG. 1: METHOD OF EXTRACTION

Transverse Section Study¹⁷:

T.S. of Leaf: The T.S. of a leaf reveals three separate regions: top epidermis, lower epidermis, and mesophyll. The upper epidermis of *E. hirta* leaves was uniform, regular, thin-walled, and covered in a thin cuticle layer. Trichomes (multicellular uniseriate or multicellular gland-like) seen on both surfaces of leaves were surrounded by stellated epidermal cells ranging in number from 12 to 14 cells at the base of each hair. Mesophyll was divided into palisade and spongy layers, which

were made up of parenchyma cells that varied in size. The palisade layer was on the adaxial side of the leaf and consisted of two rows of cells. The spongy layer was present in the cells that are spherical and subspherical, with big and small intercellular gaps. The thickness of the spongy layer was different around the midrib region than in other parts, so it had 2-6 rows of cells. The xylem elements started perfectly and consisted of many straight rows of mostly vessels. Variable parenchyma cells surrounded the xylem elements

internally. These parenchyma cells varied in size, allowing the vascular bundle to be completely surrounded by parenchyma cells. Vascular bundle phloem elements were abundant, occupying a large

portion of the vascular bundle in the shape of a semicircle. The lower epidermis is also single-layered, with rectangular cells bearing prominent trichome-like outgrowths.

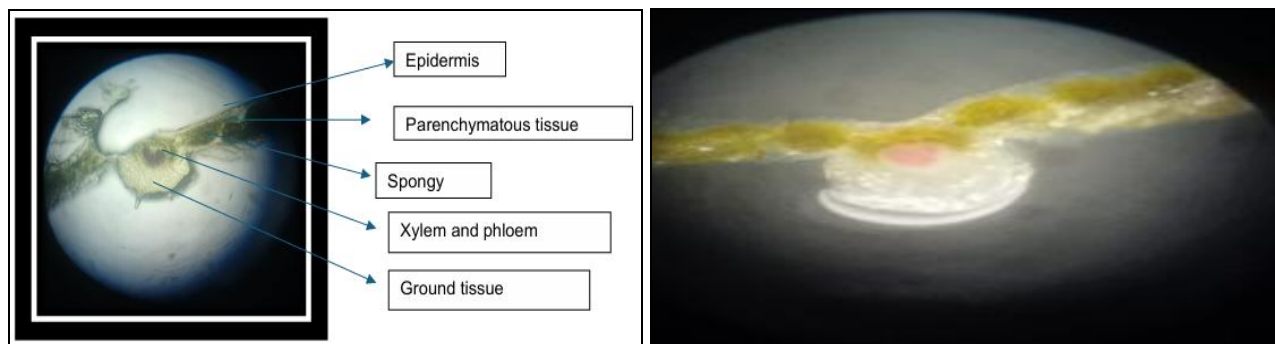


FIG. 2: T.S. OF LEAF

T.S. of Stem: The stem had a circular cross section and was covered in thick cuticle and trichomes. The epidermis was uniseriate and isodiametric, with elongated, densely packed epidermal cells containing unicellular and multicellular trichomes. Vessels and tracheids in radial rows resembled wood-treacherly elements. The xylem parenchyma was differentiated within the stem. The xylem projected clearly toward the pith, which would be single, double, or triple. The phloem was located externally and was frequently enclosed by thick

fibrous tissue similar to bundle caps. The phloem was separated by 4-6 small parenchymatous layers. The sieve elements and other phloem cells were evenly distributed between the bundle cells and xylem, and there were few Fibers, or these cell elements existed as an island between the bundle cap fibres. At the maturation stage, this species' pith exhibits a conspicuous gap or core cavity. The pith cells were large, with thin walls and distinct intercellular spaces.

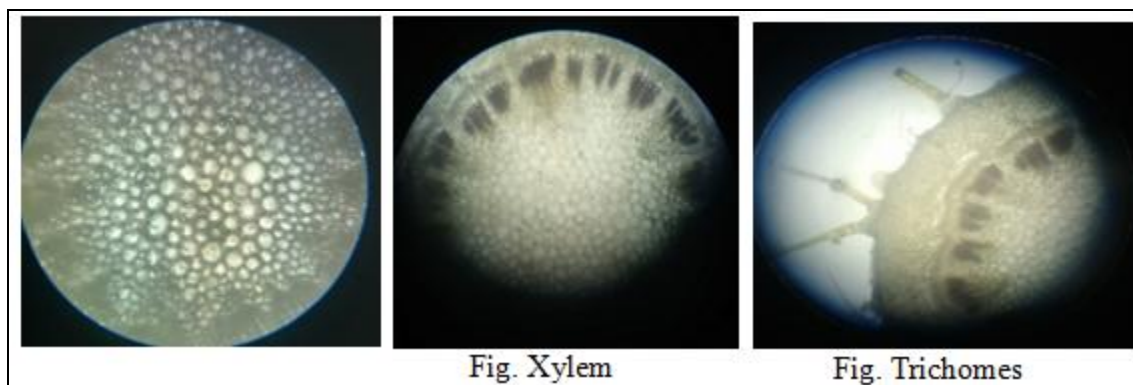


FIG. 3: T.S. OF STEM

Micrographic Study: Performed as per the Acharya D (Ref. no: 17).

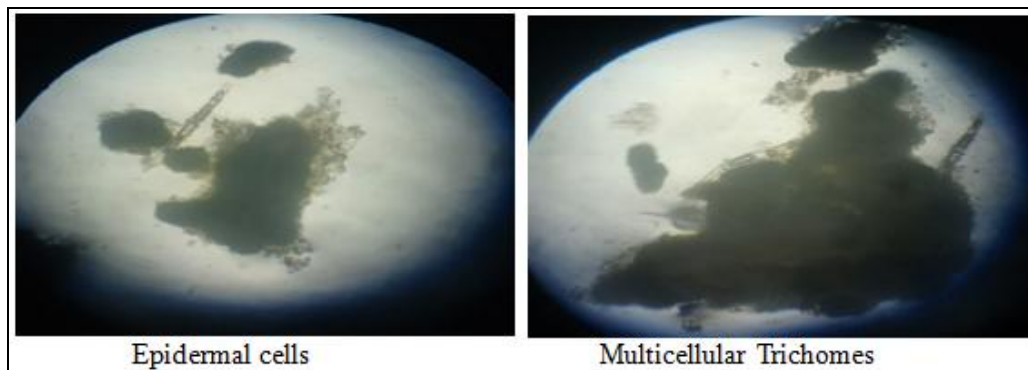


FIG. 4: POWDER MICROSCOPY OF LEAVES OF *EUPHORBIA HIRTA*

TABLE 1: ORGANOLEPTIC CHARACTER OF LEAVES OF *EUPHORBIA HIRTA*

Drug characteristics	<i>Euphorbia hirta</i> leaves
Taste	Bitter
Odour	Odourless
Colour	Ashes
Aspect	Powdered
Texture	Coarse

TABLE 2: DETERMINATION OF ASH VALUE

Sl. no.	Parts of the plant	Total ash (w/w)	Water soluble ash (w/w)
1	Leaves	7.6±0.03	3.9±0.023

Extractive Value of *Euphorbia hirta*:**TABLE 3: DETERMINATION OF EXTRACTIVE VALUE OF LEAF**

Sl. no.	Solvent	Extractive value (w/w)
1	Alcohol soluble	9.82±0.02
2	Water soluble	22.20±0.05

TABLE 4: PRELIMINARY PHYTOCHEMICAL SCREENING OF THE EXTRACTS

Sl. no.	Chemical test	Methanol extract		Aqueous extract
1	Carbohydrates			
	Molisch's test	+	-	+
	Fehling's test	+	-	+
2	Alkaloids			
	Mayer's test	-	+	+
	Wagner's test	-	+	+
3	Flavonoids			
	Alkaline reagent test	+	+	-
	Ammonium hydroxide test	+	+	+
4	Tannins			
	Ferric chloride test	+	+	+
	Gelatin test	+	+	+
5	Saponins			
	Foam test	+	-	+
6	Proteins			
	Biuret test	+	-	+
7	Steroids			
	Lieberman-Burchard test	-	+	+

TABLE 5: PERCENTAGE INHIBITION FOR ANTIOXIDANT ACTIVITY BY HYDROGEN PEROXIDE SCAVENGING METHOD

Sl. no.	Conc. (µg/ml)	Ascorbic acid	Methanolic extract	Aqueous extract
1.	20	48±0.52	49.87±0.90	39.8±2.86
2.	40	59±0.76	57.65±0.53	42.89±0.38
3.	60	66.7±0.66	62.58±0.63	46.58±0.85
4.	80	76.5±0.48	70.13±1.21	52.21±1.27
5.	100	84.49±0.79	81.97±1.23	60.38±0.47

TABLE 6: ANTHELMINTIC ACTIVITY METHANOL AND AQUEOUS EXTRACT OF LEAVES OF *E. HIRTA*

Extracts	Concentration (mg/ml)	<i>Pheretima posthuma</i> (earthworm)	
		Time for paralysis(P) in min.	Time for death (D)in min
Std. Albendazole	5	35	48
	10	26	35
	15	18	27
Methanol extract of leaves	5	60	81
	10	52	59
	15	38	57
Aqueous extract of leaves	5	81	95
	10	68	79
	15	56	64

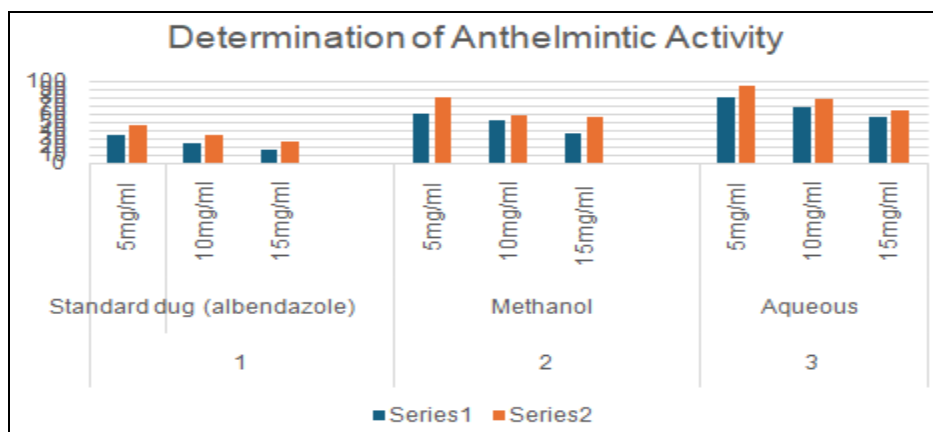


FIG. 5: GRAPHICAL REPRESENTATION OF ANTHELMINTIC ACTIVITY

CONCLUSION: The present study demonstrated the significant anthelmintic and antioxidant potential of *Euphorbia hirta*, validating the traditional uses in folk medicine. And also revealed the Microscopic, transverse section, morphological characteristics.

The methanolic extracts of *E. hirta* exhibited dose-dependent anthelmintic activity against *Pheretima posthuma*, Indicating its potential as a natural anthelmintic agent and strong antioxidant activity, scavenging free radicals and protecting against oxidative damage. This study further helps the other researcher to find the other active constituents present in the plant and which active constituent is responsible for the particular activity.

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CONFLICT OF INTEREST: No conflict of interest.

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