



**STUDY ON PHYTOCHEMICAL SCREENING AND  
PHARMACOLOGICAL PROPERTIES OF RAUWOLFIA CAFFRA**

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**ABSTRACT**

*The study investigated the pharmacological properties and phytochemical composition of the bark extract of *Rauwolfia caffra* var. *caffra*. About 1.2 kg of the powdered material was extracted exhaustively with petroleum spirit (60 -80°) in a soxhlet extractor. The preliminary phytochemical screening of the extract, was carried out using the standard procedure Preliminary phytochemical screening of methanolic and petroleum ether extracts revealed the presence of bioactive compounds such as steroids, triterpenes, higher fatty acids, phenolic compounds, and alkaloids. Pharmacologically, the methanolic extract exhibited dose-dependent relaxation effects on isolated rabbit duodenum and guinea pig ileum, antagonizing acetylcholine and histamine-induced contractions, indicating potential anti-spasmodic and anti-histaminic properties. The pharmacological screening has revealed that the methanolic extract of *Rauwolfia Caffra* has anticholinergic and antihistaminic activity against rabbit and guinea pig. Intestinal smooth muscle has relaxant effect of bark extract justifies the use of *Rauwolfia Caffra* bark as tradition remedy for abdominal pain, menstrual pain and irregular menstruation. The plant's traditional medicinal uses in Africa, including treatments for epilepsy, hypertension, and abdominal pain, are supported by these findings. This study underscores the need for further research to elucidate the mechanisms of action and identify active constituents responsible for these effects. The results contribute valuable information on the antimicrobial efficacy, antioxidant properties, and therapeutic potential of *R. caffra*, highlighting its importance in traditional healthcare practices and its potential for developing new pharmaceutical agents.*

**Keywords**

*Rauwolfia caffra*, pharmacological properties, phytochemical screening.

## INTRODUCTION

The use of plants as drugs dated back to ancient civilizations, such as Chinese, Hindus, Babylonians, Greeks, and Egyptians, who utilized traditional medicine extensively. Evidence of plant use for medicinal purposes has been found in ancient civilizations and more recent discoveries, like the fossilized peyote used by Amerindians around 7000 BC. India's herbal medicine, Ayurveda, dates back over 5000 years. Records from ancient Chinese and Egyptian texts highlight the use of herbal medicines, such as Chaulmoogra oil for leprosy, castor oil seeds, and opium poppy seeds (Ekor, 2016).

According to (Kambizi, L., & Afolayan, A. J., 2016) ,traditional medicine remains prevalent, especially in developing countries where about 75-90% of rural populations rely on herbal care. According to the (Organization, (2019).) , identified over 20,000 species of medicinal plants, acknowledging their importance. Notably, many modern drugs were initially derived from plants used by ancient societies.

Plants contain chemical constituents like alkaloids, glycosides, and essential oils, responsible for their medicinal properties. These natural compounds are often used as therapeutic agents or as blueprints for synthetic drugs. Traditional medicines are cheaper and more accessible than modern synthetic drugs, making them essential in regions with limited healthcare resources (Ncube, B., Finnie, J. F., & Van Staden, J., 2016) .

In developing countries, traditional medicine is integral to primary healthcare. There is a need to confirm and standardize the efficacy of traditional herbs, especially considering the high cost of modern drugs. Despite the importance of traditional herbal drugs, efforts to standardize it in regions like Africa are minimal, even though these areas are rich in plant species (Kambizi, L., & Afolayan, A. J., 2016) .

**Rauwolfia caffra** is a large tree in the Apocynaceae family, locally known in Nigeria as Wada (Hausa) and Awa (Yoruba). It grows up to 21 meters high and has specific features like whorled branches and gray rough bark. Found

widely in tropical Africa and South Africa, particularly in forested, swampy areas. Contains predominantly indole alkaloids. Studies have identified several alkaloids such as ajmaline, ajmalicine, and reserpine. These compounds are known for their toxic and medicinal properties (Kambizi, L., & Afolayan, A. J., 2016) . It contains Saturated and unsaturated fatty acids, with saturated ones being more common and insoluble in water. Unsaturated fatty acids, which include oleic and linoleic acids, have lower melting points due to their double bonds. Based on isoprene units (C<sub>5</sub>H<sub>8</sub>)<sub>n</sub>, they range from monoterpenes to polyterpenoids. These compounds are found throughout the plant kingdom, in essential oils, resins, and natural rubber (Semenya, S. S., & Maroyi, A., 2019) ).

Known for its alkaloid content, this plant's extracts have been studied for their potential therapeutic effects. Alkaloids like ajmaline and reserpine are notable for their medicinal properties. *R. caffra* was found to have molluscicidal activity. The methanolic extract of the dried leaves was tested against *Bulinus globosus* and the extract has shown activity with LD<sub>90</sub> of 50ppm. Also, water extract of the dried leaves of South African *R. caffra* specie has shown a similar molluscicidal activity with LC50 of 1.943mg/ml and the extract was tested against *bulinus africanus* (Omokhua, A. G., & Idu, M., 2020).

The plant was confirmed to have no antischistosomal activity after challenging *schistosoma haematobium* with 2mg/ml of ethanolic extract of the plant. The extract has shown no activity on the adult schistosoma, the miracidia, the cercariae and the ova (Omokhua & Idu, 2020). *Rauwolfia caffra* has shown cardiotoxic activity on the molluscan heart 50ppm of the methanolic extract was tried on the heart of *Archachatina marginata* and *Biophalaria glabrata* and both showed cardiotoxic response (Adewumi and Adesogan, 1986). However, the methanolic extract did not shown any antibacterial activity when tried on *E. coli* and *N.gonorrhoeae*; although it did shown a weak activity against yeast cells (Chhabra and Uiso, 1991).

## **The Ethnomedicinal Uses of Rauwolfia caffra**

Kambizi and Afolayan (2016) reports have shown that there was an average of 70 death per year as a result of ingestion of *Rauwolfia caffra* in Tanzania. Also in South Africa, the plant is used as a supplement in preparing arrow poison. However, despite the toxicity of *Rauwolfia caffra* it is widely used as a medicinal remedy of many ailments in Africa. In Vanda, the decoction of the dried bark of *Rauwolfia caffra* is used as a bath and inhalant in the treatment of epilepsy. Also, in conjunction with other plant *Peltophoron africanum*, *Rauwolfia caffra* is used for treating eye sickness by exposing the eyes to steam of the plants under a blanket (Omokhua & Idu, 2020).

Semenya & Maroyi (2019), have reported the use of *Rauwolfia caffra* as a remedy for high blood pressure in Tanzania. Also, the decoction of the plant is used in treating abdominal pain, constipation and irregular menses. The bark decoction of *Rauwolfia caffra* is also applied to the skin for treating measles urticaria and other rashes. Furthermore, the bark decoction is used externally as an insecticide to kill maggots in open wounds. It is used for treating scrofula and also as a purgative, although it is associated with abdominal pain. *Rauwolfia caffra* has also found application in treatment of pneumonia where the stem – brak decoction is used, likewise in the treatment of Rheumatism, and open wounds.

Overall, the use of traditional medicine, rooted in ancient practices, continues to be a crucial aspect of healthcare, especially in developing regions. The phytochemical and pharmacological properties of plants like *Rauwolfia caffra* highlight their significant role in treating various ailments although studies on *Rauwolfia caffra* have been done elsewhere little information exist about the Nigerian species. In this study, the antimicrobial efficacy of *R. caffra* var. *caffra* was examined using three species of bacteria namely *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*; the antioxidant properties and phytochemical potential of the plant was also examined. The results will be useful and may contribute in the development of pharmaceutical industry.

## **MATERIALS AND METHODS**

All solvent and reagents used in this work are of analytical grade. The fruits, leaves, root and bark of *Rauwolfia caffra* were collected from a forest around Kumbotso village (Kano State, Nigeria) in the month of October 2013. The plant was authenticated by comparison with a herbarium specimen at Sa'adatu Rimi College of Education Kumbotso, Kano.

After collection, the sample was dried in a hot air oven at 30°C for ten days. The dried sample was crushed and powdered. The powdered plant material was weighed and kept in a clean container and labeled as plant material.

### **Extraction of plant Material**

About 1.2 kg of the powdered plant material was extracted exhaustively with petroleum spirit (60 -80°) in a soxhlet extractor. The extract was concentrated to a brownish waxy material that was thereafter referred to as the Petroleum Ether extract (PE). The material was air – dried, and thoroughly extracted with methanol for about 48 hours. The methanolic extract was concentrated in-vacuo using rotary evaporator to dark brownish mass, subsequently (ME).

### **Preliminary Phytochemical Screening**

The petroleum ether and methanol extracts were subjected to qualitative phytochemical analysis following standardized methods (Sciences, 2020). Tests were conducted for alkaloids, steroids, triterpenes, flavonoids, coumarins, phenolics, glycosides, and saponins.

### **Preliminary Phytochemical Screening of PE**

a) Test for steroids and triterpenes: About 0.2 gm of PE was dissolved in equal amounts (0.5ml) of acetic anhydride and chloroform. Concentrated sulfuric acid (1-2ml) was added to the bottom of the tube (Leibermann-Burchard's reaction). The presence of either sterols or triterpenes would be inferred when a brownish – red or violet ring is formed at the contact zone of the two liquids.

- b) **Test for Carotenoids:** About 10ml of PE was evaporated to dryness and a saturated solution of antimony trichloride in chloroform (2-3 drops) was added (Carr price's reaction). A color change to blue then later to red, indicates the presence of carotenoids.
- c) **Test for higher fatty acids:** The alkaline aqueous solution of the extract was exhaustively extracted with ether and acidified with concentrated hydrochloric acid (pH 3-4). The presence of higher fatty acid is indicated when the acidic solution turns opalescent.
- d) **Test for basic alkaloids:** About 0.2gm of PE was dissolved in 1.5ml of 2% HCl. The solution obtained was then divided into three equal portions in three test tubes. One of the test tubes was considered as a reference while to the two tubes, 2-3 drops of Mayer's and Dragendorff reagent were respectively added. An opalescence or precipitate (yellowish, white with Mayer's and yellowish reddish with Dragendorff reagent) indicates the presence of alkaloids.
- e) **Test for flavone aglycones (Shibata's reaction):** About 3 ml of PE was evaporated until a small residue was obtained. The residue was dissolved in 1-2 ml of 5% methanol while hot. Magnesium chips and 4-5 drops of concentrated HCl were added. A red or orange coloration indicates the presences of flavonic-glycoside.
- f) **Test for emodols (anthraacenoside aglycones):** 3ml of PE was transferred to a test-tube and 1 ml of 25% ammonia solution was added and shaken (Borntrager's reaction). A red color indicates the presence of emodols.
- g) **Test for coumarins:** 0.2gm of PE was dissolved in hot water, after cooling the solution was divided into two test tubes. One contains the reference, while the aqueous solution in the second tube was made alkaline with 0.5 ml of ammonia solution (10%). The occurrence of an intense fluorescence under Ultraviolet (UV) light indicates the presence of conmarins and its derivatives.

## **Preliminary Phytochemical screening of ME**

a) **Test for phenolic compounds:** 0.2 gm of ME was dissolved in water (2-4 ml) and diluted solution of ferric chloride (about 2-3 drops) was added. The occurrence of a blue- black color or a greenish – black color indicates the presence of phenolic compounds.

b) **Test for reducing compound:** 0.2gm of the methanol extract was diluted with water (about 2-4ml) and Fehling I & II solution were added and heated. A brick- red precipitate denotes the presence of reducing compounds.

c) **Test for alkaloidal salt:** About 20 ml of ME was evaporated to dryness on a boiling water – bath approximately 5ml of 10% HCl was added to the residue containing alkaloidal salts of organic acid. The alkaloids were converted to salts of mineral acid. From the aqueous solution, the alkaloids were then precipitated by adding 10% ammonia solution. Then the alkaloids were extracted with chloroform. The chloroform solution was then evaporated to dryness. The residue was divided into 3 test tubes. One tube was kept as a reference while to each of the other two tubes, 2 drops of Dragendorff and Meyer’s reagents were added. The presence of alkaloidal salts was indicated by change of coloration to reddish precipitate for Mayer’s reagent.

## **Hydrolysis of ME**

About 25ml of ME was taken and 15ml of 10% HCl was added by refluxed for 30 minutes after cooling, the solution was 3 time extracted with ethyl ether and dehydrated with anhydrous sodium sulfate resulting in ether and aqueous solution. The ether extract was used for the following test: -

### **a) Test for anthracenosides**

4ml of the ether extract was concentrated in 2mls and then ammonia solution (25%) was added (1-2ml) by shaking. A cherished color of the alkaline solution indicates the presence of aglycones of anthracenosides (Borntragers’ reaction).

### **b) Test for coumarin derivatives**

5ml of the ether extract was evaporated to dryness, the residue was dissolved by heating in 1-2ml of water. The aqueous solution was divided into two equal volumes in two test tubes. To one of the tubes, 0.5ml of 10% ammonia solution was added. The other tubes serve as a reference. The occurrence of a blue or green fluorescence under Ultraviolet (UV) light indicates the presence of coumarins.

### **c) Test for triterpenes and steroid glycoside (Liebermann –Burchard’s reaction):**

10ml of the ether extract was evaporated to dryness, the residue was dissolved successively in acetic anhydride (0.5ml) and chloroform (0.5ml). The solution was transferred to a dried test tube. By means of a pipette, concentrated  $H_2SO_4$  (1-2ml) was added at the bottom of the tube. At the separating level of the two liquids a reddish-brown ring was formed. The upper layer turning to greenish – blue indicates sterols and triterpenes.

### **d) Test for cardenolides (Kedde’s test)**

The residue of ether extract by evaporating the ether extract (4ml) was dissolved in methanol (1-2ml); alcoholic solution of KOH (1-2ml) and alcoholic solution of 3 3-5 dinitrobenzoic acid (3-4 drops) were added. Disappearing violet coloration would indicate the presence of cardenolides.

## **RESULTS:**

### **Preliminary Phytochemical Screening of R. Caffra Bark**

The results of preliminary phytochemical screening of PE showed it to contain higher fatty acids, sterols, and triterpenes while basis alkaloid, coumarins, flavonoids, and emodols were found to be absent (Table 1).

Table 2 summarizes the result of the preliminary phytochemical screening of ME, where phenolic compounds, reducing compounds, and alkaloidal salts were found to be present in the plant bark extract.

ME was shown not to contain glycosides. Table 3 summarize the results obtained from the preliminary phytochemical screening of the hydrolyzed ME.

Alkaloids, steroids, and triterpenes were present while coumarins and flavonoids in general were absent in the hydrolyzed ME.

**Table 1: Preliminary Phytochemical screening of PE**

<b>Chemical group</b>	<b>TEST</b>	<b>OBSERVATION</b>	<b>INFERENCE</b>
<b>Alkaloidal Base</b>	Dragendorff reagent	No precipitation	Basic alkaloids absent
<b>Alkaloidal Base</b>	Mayer's reagent	No precipitation	Basic alkaloids absent
<b>Alkaloidal Base</b>	Wagner's reagent	No precipitation	Basic alkaloids absent
<b>Sterols and triterpenes</b>	Liebermann Burchard test	Greenish coloration	Sterols and/ or triterpenes present
<b>Coumarins</b>	Aq. Extract + NH <sub>3</sub> and observed under U.V. light	No fluorescence	Coumarines absent
<b>Higher fatty acids</b>	Alkaline aq. Solution of extract, extracted with ether and acidified with conc HCl (pH 3-4)	Opalescence of acidic aq layer	Higher fatty acids present
<b>Flavones aglycone</b>	Shinoda test	No red or orange coloration observed	Flavonoids absent
<b>Emodols</b>	Bortrager's reaction	Absence of red coloration	Emodols absent

**Table 2: preliminary Phytochemical screening of ME**

<b>Chemical Group</b>	<b>Test</b>	<b>Observation</b>	<b>Inference</b>
<b>Phenolic compounds</b>	Ferric chloride solution extract	Bluish – black coloration observed	Phenolic compounds present
<b>Reducing compound</b>	Fehling I & II solution	Brown precipitate	Reducing compound present
<b>Alkaloids</b>	Fragendorff Reagent	Orange – red precipitate	Alkaloid salt present
<b>Alkaloids</b>	Mayer's reagent	White – yellowish precipitate	Alkaloidal salt present
<b>Alkaloids</b>	Wagner's reagent	Reddish precipitate	Alkaloidal salt present

<b>Group</b>	<b>Test</b>	<b>Observation</b>	<b>Inference</b>
<b>Coumarin derivatives</b>	Aq extract + 10% NH <sub>3</sub> and observed under U.V light	No fluorescence UV	No coumarins detected
<b>Steroids</b>	<b>and</b> Liebermann Burchard	Brownish ring at the interface and bluish coloration of the upper	Steroids and / triterpenes

<b>triterpenes</b>	reaction	layer	present
<b>Flavones glycoside</b>	Shinoda test	No red coloration or orange coloration	Absence of flavonols and flavanones
<b>Alkaloids</b>	Dragenforff reagent + extract	Orange red precipitates	Alkaloids present
<b>Alkaloids</b>	Wagner's reagent + extract	Reddish precipitate	Alkaloids present
<b>Anthracenosides</b>	Ether extract + 25% NH <sub>3</sub>	No cherished red coloration	Absence of anthracenosiders

**Table 3: Preliminary phytochemical screening of the hydrolysed ME**

<b>Anthracenosides</b>	<b>Ether extract + 25% NH<sub>3</sub></b>	<b>No cherished red coloration</b>	<b>Absence of anthracenosides</b>
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## Pharmacological screening

### Effect of ME on Isolated Rabbit Duodenum

Table 4 summarizes the response of different doses of acetylcholine on isolated rabbit duodenum. The tissue showed normal response to the agonist. These contractions were antagonized by atropine. Similarly, ME was observed to reverse these Ach-induced contraction dose dependently. ME was observed to relax rhythmic contraction on the isolated rabbit duodenum.

**Table 4: Response of Ach on Isolated Rabbit Duodenum**

Conc. Ach (µg/ml)	Response (cm)	Log Dose	Log Dose+3	% Response
0.05	1.5	-2.3	0.7	15
0.01	7.8	-2.0	1	79
0.02	9.0	-1.7	1.3	90
0.04	9.9	-1.4	1.6	100

**Table 5: Effect of Ach in the Presence of Various Doses of Atropine (Antagonist)**

Conc. (µg/ml)	Response (cm)	Log Dose	Log Dose +2	% Response
0.04	0.2	-1.4	0.6	13
0.08	0.7	-1.1	0.9	47
0.16	1.5	-0.8	1.2	100

**Table 6: Effect of Extract on the Isolated Rabbit Duodenum**

Conc. Adm. (µg/ml)	Response	Log Dose	% Response
200	1	2.3	100
400	0.6	2.6	60
800	0.1	2.9	100

**Table 7: Effect of Ach (0.02 µg/ml) and ME on Isolated Rabbit Duodenum**

<b>Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Response (cm)</b>	<b>Log Dose</b>	<b>% Response</b>
<b>100</b>	1.3	2.0	100
<b>200</b>	1.3	2.3	100
<b>400</b>	1.2	2.6	92
<b>800</b>	0.9	2.9	69
<b>1600</b>	0.7	3.2	54
<b>3200</b>	0.3	3.5	23
<b>6400</b>	0	3.8	0

### **Effect of ME Isolated Guinea pig Ileum**

Histamine showed as dose dependent contraction of isolated guinea ileum (Table 8). While ME showed a relaxant activity on the isolated ileum. ME antagonized the histamine induced contraction of the isolate quinea pig ileum (Table 8) shows the responses of isolated guinea pig ileum to doses of histamine and ME.

**Table 8: Effect of Histamine on Guinea Pig Ileum**

Conc. ( $\mu\text{g/ml}$ )	Response (cm)	Log Dose	% Response	Log Dose +3
0.004	0.6	-2.4	9	0.6
0.008	1.2	-2.1	18	0.9
0.016	2.2	-1.8	34	1.2
0.032	6.5	-1.5	100	1.5

**Table 9: Effect of Histamine (0.25  $\mu\text{g/ml}$ ) and ME**

Conc. ( $\mu\text{g/ml}$ )	Response (cm)	Log Dose	% Response
800	2.7	2.9	100
1600	1	3.2	37
2400	0.3	3.4	11
3200	0	3.5	0

## DISCUSSIONS

### Phytochemical Screening

#### *Petroleum Ether Extract*

The petroleum ether extract was found to contain steroids and triterpenes as inferred from the positive Liebermann Burchard reaction. Also, higher fatty acids were found to be present in the non-polar extract. However, basic alkaloids were not found despite the high alkaloidal content of the plant. Also, flavonoids, emodols, and coumarins were found to be absent in the non-polar extract.

#### *Methanolic Extract*

The preliminary phytochemical screening of the methanolic extract has revealed the presence of alkaloids. Positive ferric chloride test has shown the presence of phenolic compounds. But the hydrolysis of the methanolic extract has revealed the absence of glycosides.

These results are consistent with prior studies reporting high levels of alkaloids and phenolic compounds in *R. caffra* (Tshikalange, T. E., McGaw, L. J., & Eloff, J. N., 2018).

## **Pharmacological Screening**

### **Effect of Extract on Rabbit Duodenum**

From the result, it can be seen that Acetylcholine has shown the usually expected rhythmic contractions of the rabbit duodenum and the contraction are also the expected dose dependent.

Acetyl choline, a mediator of the central synaptic transmission that mediates the transmission of impulse from the postganglionic termination of the parasympathetic nerves to the affected organ. It has muscarinic activity on the rabbit smooth – muscle thereby stimulation the smooth muscle and consequently causing contractions of the smooth muscle.

Atropine, a parasympathetic blocker antagonizes the action of Acetylcholine by preventing the access of acetylcholine to muscarinic receptors. The tone of intestinal smooth muscle is inhibited by atropine, and it can be seen from the experiment that atropine antagonizes, the action of acetylcholine.

Also, the extract of R. Caffra sample has shown antagonism to Acetylcholine which is dose- dependent. The mode of action of the extract could be due to competitive antagonism contrary to the effect shown by atropine 0.025ml of the extract (100mg/ml), has shown no activity in the presence of Acetylcholine. However, the extract alone has produced a direct and non-dose dependent relaxation of rabbit duodenum. The experiment has shown that 6500 µg/ml is required to overcome the muscarinic activity of 0.04µg/ml of acetylcholine completely and also abolish any contractile tone of the smooth muscle.

### **Effects of the Extract on the histaminic Activity of Guinea Pig Ileum**

Histamine causes contraction of smooth muscles through its action on histamine receptors. The guinea pig ileum is very sensitive to histamine due

to abundance of histamine receptors; thus, it is usually used to screen drugs or extract for anti-histaminic activity.

Histamine has shown a dose dependent contraction of guinea pig ileum. However, the extract alone has shown no-dose dependent relaxation of the guinea pig ileum. This could be due to antagonistic action of the extract to the endogenous histamine by blocking the receptors, (Table 8).

ME antagonized histamine induced contraction of isolated guinea pig ileum. The maximum concentration of ME that completely blocks the histamine induced contraction was found to be 3200 µg/ml, (table 9)

The anti-cholinergic and anti-histaminic activity of *R. caffra* bark can be the basis for its use by traditional healers as a remedy for abdominal pain and itching rashes like those of measles. This confirms its anticholinergic and antihistaminic potential as indicated by (Ekor, 2016; Omokhua & Idu, 2020).

## CONCLUSION

It can be concluded from the study that *Rauwolfia Caffra* contain alkaloids, sterols, triterpene, phenolic compounds, higher fatty acid and sesquiterpenes. Preliminary phytochemical study has indicated the presence of terpenoidal compound in the plant. Numerous alkaloids (over 40) were reported to have been isolated from the plant however, not a single fatty acid was reported to have been isolated.

The pharmacological screening has revealed that the methanolic extract of *Rauwolfia Caffra* has anticholinergic and antihistaminic activity against rabbit and guinea pig. Intestinal smooth muscle has relaxant effect of bark extract justifies the use of *Rauwolfia Caffra* bark as tradition remedy for abdominal pain, menstrual pain and irregular menstruation. The antihistaminic effect of the extract can also explain the use of *R. Caffra* bark for treating measles, urticaria and other skin rashes.

The study supports the ethnomedicinal use of *R. caffra* in managing spasmodic and allergic conditions. Phytochemical analysis confirmed the

presence of active constituents, while pharmacological assays showed antispasmodic and antihistaminic activity. Further studies are needed to isolate specific compounds and evaluate their therapeutic potential.

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