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Potentials of *Amaranthus Spinosus* and *Xylopia Aethiopica pods* Extracts for Tooth Silkworm Removal

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Highlights:

Point-out that *amaranthus spinosus* and *xylopia aethiopica pods* extractives demonstrated analgesic potential across ages and sexes

Shows the higher efficacy associated with *xylopia aethiopica* and its alkaloid-rich extracts.

Identifies phytochemicals and the observed survyed intrested outcome supported anti-infection/anti-inflammatory.

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Abstract: Toothache has left no one undisturbed at various stages in life. It is worse in two extreme ages: childhood and old age. The extracts of Amaranthus spinosus and Xylopia aethiopica Pods showed analgesic potential across ages and sexes in the removal of a causative toothache known as silkworm. Xylopia aethiopica pods extract yielded higher (1.5 g) compared to Amaranthus spinosus whole plant (0.6 g), with both samples having high contents of alkaloids; 85.02 ± 0.22 for the pods and 50.82 ± 0.05 for the leaves. The phytochemical analysis revealed the presence of alkaloids and saponins. Phenolic, flavonoid and Cardiac glycosides in Xylopia aethiopica pods and Amaranthus spinosus. All patients had complete relief and good sleep after the mouth steam, which indicated that extractives possess an anti-infection (bacterial) or an anti-inflammatory property. The silkworm removal by respondents (A. C & F) acts as a painkiller, which could be related to the characteristic nature of the phyto-constituents in the combined extracts. Only one exceptional case in (A) showed sucked out blood. Thus, silkworm removal using the combined extractives was shown to be a reliable alternative to orthodox medicine for comfort and pain relief.

Keywords: *Amaranthus spinosus*, *Xylopia aethiopica* Pods, Tooth silkworm, phytochemical analysis.

Introduction

Plants are known to have been existed before the creation of mankind as their main sources of food, shelter, contributed largely to human health and wellbeing. In overcoming the dental problems, especially tooth-ache caused by bacteria's and worms' different plant has been investigated and two of its types are *Amaranthus spinosus* and *Xylopia aethiopica* Pods. Silkworm as a life science economic insect used as model in scientific researches (Meng et al., 2017), it also support agricultural roles (Banno et al., 2010). *Amaranthus spinosus* as consumable leave (Anjali Ganjare and Nishikant Raut, 2019; Atchou et al., 2021) and as fish feeds (Patalinghug et al., 2022) is commonly refers to as prickly amaranth, a noxious weed, spiny pigweed or spiny amaranth, is found in the tropics of united states of America, at temperate region in Japan, spread to most region in Africa (Jimoh et al., 2022) especially in Kogi state. *Amaranthus spinosus* contain phytoconstituents (Jhade et al., 2011; Kar & Bhattacharjee, 2022; Nasir et al., 2020; Sharma et al., 2021) such as amaranthine, isoamaranthine, hydroxycinnamates, quercetin and kaempferol glycosides (Stintzing et al., 2004), α-spinasterol

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(Billah et al., 2013), rutin and hydroxycinnamic acid derivatives (Karamać et al., 2019), Quercetin and Gallic acid (Swarnakumari et al., 2021), fatty acid (14E, 18E, 22E, 26E) – methyl nonacosa-14, 18, 22, 26 tetraenoate and β-sitosterol (Mondal et al., 2015). It is used in the management of large range of diseases (Baral et al., 2011; Manu et al., 2012; Peter & Gandhi, 2017) and act as antimicrobial (Yahya et al., 2021), anti-inflammatory (Rajasekaran et al., 2014), and antioxidant (Amabye, 2016) haven antioxidant property (Sarker & Oba, 2019). It rediscovered for great therapeutic potentials (Peter & Gandhi, 2017). It nutritional value was studied (Anjali Ganjare and Nishikant Raut, 2019) while its serves as meal (Adewolu & Adamson, 2011). As such, different extraction method were employed (Balkumar, 2022). It possess cytotoxic activity and as a good cancer apoptosis (Atchou et al., 2021; Indumathy & Pharm, 2016; Thoppil & Prajitha, 2017). Tropical and subtropical Africa are very rich in angiosperm of the family Annonaceae with genus *Xylopia* of at least 150 species (Hwang & Simon, 2020). *Xylopia aethiopica pods* was one of the well use species as reproductive stimulated hormone (Okonkwo et al., 2021). Their extracts are found effective in the treatment of microbial diseases such as malaria cough, bronchitis and uterine fibroid (Fetse et al., 2016) supporting it phytochemical analysis (Aguoru & Olasan, 2016). It reduces yam tuber fungi infection (Okigbo & Nmeka, 2005). Inhibits commonest microbes, Escherichia coli and bacillus cereus (Ilusanya, O.A.F., Odunbaku, O. A., Adesetan, T.O., Amosun, 2012) which contaminates food. Its hyperglycemic effect was not left out (Mohammed et al., 2016). *Xylopia aethiopica* act as antioxidant(Okigbo & Okoli, 2020) and stabilizer used in food industry (Babarinde & Adegoke, 2015). It essential oil act as mild steel anticorrosion due to the presence of heteroatoms present (Okafor & Apebende, 2014). Mainly alkaloids (Fetse et al., 2016). Other identified phytochemicals include saponins, sterol, carbohydrates, glycosides, tannins, phenol and aromatic volatile oils. Their use in traditional management for dental diseases is hardly reported. In addition, world health organization have estimated caries of permanent teeth problems to about two billions and children of primary teeth of five hundred and twenty millions by global burden diseases study (Burden, 2022). As such, both plant extracts were evaluated for the removal of silkworm using volunteered affected patients.

Plant Materials

The *Amaranthus spinosus and Xylopia aethiopica pods* were collected during Okene market, Okene Local Government Area of Kogi State, Nigeria. The plants were identified and confirmed at Biological Department, Federal College of Education Okene Kogi State by Mrs. Aniama S.O.A. a botanist. The plant materials were authenticated at Forestry Research Institute of Nigeria Ibadan through comparison with the voucher specimen by Mr. Michael. The plant samples were collected, and air dried at room temperature for one month and turned to powder to be used for phytochemical analysis. The collected samples were shown in Figure 1.

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Figure 1: (a) *Xylopia aethiopica* pods (b) *Amaranthus spinosus* and (c) Fermented corn water

Extraction of *Xylopia aethiopica pods* and *Amaranthus spinosus* for Phytochemical Analysis

The dried powdered sample of *Xylopia aethiopica pods* and *Amaranthus spinosus* (50 g) each were macerated with methanol (MeOH) for three days. The extracts of various plants were filtered, after then similar extraction process was repeated twice using fresh methanol. The filtrate was concentrated in *vacuo* at 40^{o}C using rotary evaporator to yield respective crude extracts for phytochemical analysis.

Preliminary Phytochemical Screening Method

The standard procedures to identify constituents described (Sivanandham, 2015) were used for detection of various phytochemicals. These were followed by qualitative chemical test to give idea regarding nature of constituents present in the *Xylopia aethiopica pods* and *Amaranthus spinosus* follows.

Tests for Simple Sugar (Fehling's Test)

1 mL Fehling's A solution and 1 mL of Fehling's B solution were mixed. 1 mL from the solution was added to 3-5 mL test solution and immersed in water bath for 5-10 mins. First a yellow, then brick red precipitate was observed.

Test for Proteins (Biurret Test)

To the small quantity of extract 1-2 drops of Biurret reagent was added. Formation of violet colour precipitate showed presence of proteins.

Test for Anthraquinone Glycosides (Borntrager's Test)

To the 3 mL of extract, dil. H_2SO_4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well, and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of anthraquinone glycosides.

Test for Cardiac Glycosides (Keller-Killiani Test)

To the 5 mL of extract, 1 mL of conc. H_2SO_4, 2 mL of Glacial acetic acid and 1 drop of $FeCl_3$ solutions was added. Appearance of brown ring shows the presence of cardiac glycosides.

Test for Coumarins

To the 2 mL of extract 10% NaOH was added and shake well for 5 mins. It shows yellow colour (Lončar et al., 2020).

Test for Saponins (Foam Test)

To 1 mL extract 20 mL distilled water was added and shakes well in measuring cylinder for 15 mins. Then 1cm layer of foam was formed.

Tests for Quinone

To the 2 mL of extracts, add conc. H_2SO_4 acid carefully and shake well for 5 mins. It shows red colour.

Test for Steroids (Salkowski Test)

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To 2 mL of extract, 2 mL of chloroform and 2 mL of conc. H_2SO_4 was added. The solution was shaken well. As a result, chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Test for Alkaloids (Hager's Test)

To the 2-3 mL of filtrate, 1 mL of dil. HCl and Hager's reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

Test for Flavonoids (Lead Acetate Test)

To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

Test for Phenolics and Tannins ($FeCI_3$ Solution Test & Lead Acetate Test)

On addition of 5% $FeCl_3$ solution to the extract, deep blue-black colour appeared.

On addition of lead acetate solution to the extract white precipitate appeared.

Procedure for Total Phytochemical Contents

Total Alkaloid Content

To determine the total alkaloid content, the procedure used in (Prajapati et al., 2024) was modified and adopted as followed. The chemicals used were 50 mg/L bismuth nitrate, 1 M thiourea, 1 M diluted hydrochloric acid, and 5% disodium sulphide. The standard curve was obtained with bismuth nitrate pentahydrate stock solution. Serial dilutions of standard stock were made by pipetting out 0, 0.2, 0.4, 0.6, 0.8 and 1 mL respectively. Standard stock of 50 mg/L Bismath nitrate pentahydrate was taken in the test tubes with 0.2 mL of test samples. Five millilitres of thiourea solution was added, and water was used to make up the volume to 10 mL. Absorbance was read at 435 nm. For the herbal sample, the samples were maintained at pH 2-2.5 with 1 M hydrochloric acid. Subsequently, 2 mL of Dragendorff's solution was added, and the solution was centrifuged for 10 minutes at 1500 rpm for precipitation. After centrifugation, 2 mL of disodium sulphide solution was added, and a brown-black precipitate was formed. Two millilitres of concentrated nitric acid was then added, and the volume was made up to 10 mL with water. One millilitre of solution was discarded, and 5 mL of thiourea was added. The absorbance was read, and the concentration was determined based on the standard curve.

Total Saponin Content

To determine the total saponin content, the vanillin sulphuric acid method (Wirman et al., 2024) was used. The chemicals used were 8% (w/v) vanillin, 72% (v/v) sulphuric acid, and 0.5 mg/ mL diosgenin. A quantity of 0.25 mL sample was added to 0.25 mL of 8% vanillin in ethanol and 2.5 mL of 72% sulphuric acid. The solutions were incubated for 15 minutes at 60°C. The absorbance of the standards was measured at 765 nm. A standard curve was plotted against diosgenin, and the concentration was hence determined by extrapolation.

Total Flavonoid Content

To determine the total flavonoid content (Bhaigyabati et al., 2014; Ci & Indira, 2016). The aluminium chloride colorimetric assay was carried out, and the chemicals used were 10% aluminium chloride, 5% sodium nitrite, 10 mg of Quercetin and 1 M sodium hydroxide. One millilitre test sample and 1 mL of standard Quercetin solution were aliquoted into test tubes, and 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution were added into each tube. After 5 minutes, 0.3 mL of 10% aluminium chloride was added. At the sixth minute, 2 mL of 1 M sodium hydroxide was added, and the volume was made up to 10 mL with distilled water and mixed well. An orange colour appeared,

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and the absorbance at 630 nm was measured for each sample. The concentration was determined by using the standard curve against Quercetin.

Total Phenolic Content

To determine the total phenolic content (Karamać et al., 2019), the chemicals used were Folin-Ciocalteu's (FC) reagent, 8% saturated sodium carbonate solution, and 25 mg/50 mL gallic acid. The total phenolics of the extract was determined using FC reagent. Standard readings were made using a spectrophotometer at 765 nm against a black reagent. A quantity of 0.2 mL of the test sample was mixed with 0.6 mL of water and 0.2 mL of FC reagent. After 5 minutes, 1 mL of sodium carbonate was added to the solution, and the volume was made up to 3 mL with distilled water. The solutions were incubated for 30 minutes at room temperature and then centrifuged. The absorbance of each sample was measured. The phenol concentration was calculated as gallic acid equivalent based on a standard curve.

Sample Extraction for the Evaluation of Silkworm Removal

The dried powdered sample of *Amaranthus spinosus* (5 g) and *Xylopia aethiopica pods* (2 g) each were boiled in a small pressure pot with fermented corn water of 50 cl for thirty minutes. The steam and homogeneous extracts were used for the evaluation.

Evaluation Procedure

Both the steam and the homogeneous extracts was used. The top cork of the pot was gently released, and each patient takes their mouth very closer to the steam. Steaming process were continued for 3-5 minutes morning and evening for two days. After which the third day, the extracts were used to gurgle their tooth warmly, morning and evening. This method was used for detection of the presence of silkworm in the tooth and physically confirm bacterial infection for each of the patient.



Figure 2: Mouth Steam procedure

Experimental Design

In this study, seven patients were involved, four males and three females. No variables in terms of colours and height were considered. They are above maturity age. The dependent respondents considered for evaluating the steam and the extracts procedure were design as follows: *(A)*: Maimuna (> 40 years), *(B)*: Sakeenah, (> 30 years*), (C)*: Hauwa (> 45 years*), (D)*: *(E)*: *(F)*: and *(G)*. Jugson (> 38) The unexplained effects for the tooth pain relived within few minutes of mouth steaming process were noted randomizing in the order of the experiments. Silkworm removal on each occasion was snapped and

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documented. The names of independent respondents for experimental design were coded and decoded terms as agreed.

RESULTS AND DISCUSSIONS

The yield of extraction of *Xylopia aethiopica pods and Amaranthus spinosus* using maceration is shown in **Table 1**. *Xylopia aethiopica pods* extract yielded higher (1.5 g) compared to *Amaranthus spinosus* whole plant with (0.6 g) indicated pod sometimes have high extractives to plant matrix.

Table 1: Physical Parameters of *Xylopia aethiopica pods and Amaranthus spinosus*

S/No	Extractives	Weight	Colour	Texture
1	*Xylopia aethiopica pods*	1.5 g	Yellow	Viscous
2	*Amaranthus spinosus*	0.6 g	Brown	Solid

Alkaloid, a very important phytochemical constituent of plants, showed it presence in both *Xylopia aethiopica pods and Amaranthus spinosus*. Both flavonoid and Cardiac glycosides behaved in the same tested procedure in terms of appearance formation as shown in **Table 2**. Saponins and phenolic were also detected in the tested extractives from the plant. Thus, Alkaloid, Saponins. Phenolic, flavonoid and Cardiac glycosides were all found to be present in *Xylopia aethiopica pods and Amaranthus spinosus*. These could refer to the possible bioactive constituents, Accumulation of these phytochemicals in both plants could enhance their pharmacological activities in terms of expelling the silkworm from their comfortable tooth holes. The harshness and mouth wears of the extractives when used by the patient to gurgle their mouth could also be attributed to the same phytochemical found in both plants. Fixed oil/fats. Proteins. Anthraquinone, Quinone and coumarins were completely not detected as shown in **Table 2**. It is possible that they are absent or present at a lower than detectable limit. All methods for the analysis of carbohydrates and reducing sugar showed their presence. Therefore, the plant under study possess bioactive compounds with specific medicinal values (Priyadarsini, 2021) such as anti-neurological, anti-metabolic, wound healing and anti-cancer properties (Maroyi, 2019). The total phytochemical content determination is based on the suitability of screening purpose. These depend majorly on apparent changes in colouration. The yellow colour change as FC reagent which contain phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolbdic ($H_3PMo_{12}O_{40}$) acid oxidizes tungsten and molybdene to a clearly dark blue complex (Chilian et al., 2016) as found in catalyzed hydrolysis of cellulose to glucose under hydrothermal condition (Ezugwu et al., 2020). The formation of acidic complex at either C-3 or C-5 and C-4 hydroxyl group with flavanols and flavones were observed in the aluminum chloride assay. The A- or B-rings of flavonoids formed labile complex with orthodihydroxyl groups therein at maximum absorbance ranges. Thus, the 415 nm was used as the experimental absorbance measurement. The *Xylopia aethiopica pods* extract showed high yields of extractives compared to *Amaranthus spinosus* as shown in **Table 1** with their various physical properties. This result also corresponds to their total phytochemical percentage with *Xylopia aethiopica pods* haven flavonoids, alkaloids, saponins and phenols compared to *Amaranthus spinosus* as depicted in **Table 3** and as it supported in

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their higher crude matrix reported (Mojisola Adebimpe Fategbe et al., 2021).

Table 2: Preliminary Screening of *Xylopia aethiopica pods and Amaranthus spinosus*

s/no	Constituent	Chemical	*Xylopia aethiopica pods* / *Amaranthus spinosus*	Observation
1	Alkaloids	Hager's Reagent	XAP	+++
			AS	+++
		Dragendroff's Reagent	XAP	+++
			AS	+++
		Mayer's Reagent	XAP	+++
			AS	+++
		Wagner's Regent	XAP	+++
			AS	+++
2	Carbohydrate & reducing sugar	Fehling's Regent	XAP	+
			AS	-
		Benedict's Regent	XAP	+
			AS	+
		Molisch's Regent	XAP	+
			AS	+
3	Sterols	Salkowski Reagent	XAP	+
			AS	-
4	Saponins	Foam	XAP	+++
			AS	+++
5	Phenolics	$FeCI_3$ Sol.	XAP	+
			AS	+++
		Lead Acetate	XAP	+
			AS	+++
6	Fixed oil & fats	Spot test	XAP	+
			AS	-
7	Proteins	Biurret Reagent	XAP	-
			AS	-
		Million's Regent	XAP	-
			AS	-
8	Anthraquinone glycosides	Borntrager's Reagent	XAP	-
			AS	-
9	Cardiac glycosides	Keller-Killiani Reagent	XAP	-
			AS	+++
10	Flavonoids	Lead Acetate	XAP	+++
			AS	+++
		Extract + NH_3	XAP	+++
			AS	+++
11	Quinone	Extract + Conc. H_2SO_4	XAP	+
			AS	-
12	Coumarins	Extract + 10% NaOH	XAP	-
			AS	-

Key: NB: + -Present; ++ - Deeply Present; +++-Very deeply present

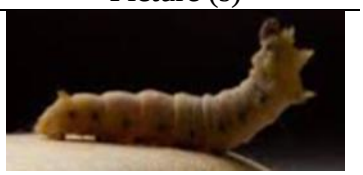
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Table 3: The Composition of Total Phytochemicals Percentage (%)

Extractives	**Flavonoids**	**Alkaloids**	**Saponins**	**Phenols**
Xylopia aethiopica pods	83.22 ± 0.09	85.02 ± 0.22	69.14 ± 0.30	66.57 ± 0.25
Amaranthus spinosus	50.88 ± 0.06	50.82 ± 0.05	18.95 ± 0.55	19.27 ± 0.65
P value				p>0.05

Means with the same letters in the same column are not significantly different at P>0.05 using Duncan's Multiple Range Test (DMRT)

Table 4. Observation and Comments for the Independent Respondents

S/L	Comments	Picture (s)
(A)	It causes me headache, fever, swollen gum and sometimes swollen jaw, when I suck it, it brings out blood. The pain is not continuous. Just for some days or weeks. I can't take cold water and oranges. Doing the mouth steam has put an end all the mention symptoms for the first day. A small silkworm came out from my mouth alive.	
(B)	A serious pain my mouth down to the head with swollen jaw. The pain prevents me to sleep both day and night. Conducting the mouth steam, complete pain relief was observed and very okay since that time.	
(C)	One side of my mouth swollen (left side), severe pain and jumping headache. I can't bite and chew food with it. I usually found small, stacked food in between my teeth. Very difficult for me to sleep. Trying the mouth steam changes my condition but one silkworm came out from the point of pain,	
(D)	I thought it was an evil people breaking my head due to the vibrational pain from the mouth to the head and vice-versa. After two days of this mouth steaming. I know believe that is ordinary sickness because all the pain goes off.	
(E)	The pain is from my mouth to the neck and to the head. Inside my left mouth swollen. I can eat again due to the discomfort and pain. The mouth steam is wonderful. The pain stop immediately I finished steaming my mouth.	
(F)	Ibuprofen tablet can only reduce the pain but cannot stop it completely. The pain and my swollen left side was on fire. An instant pain disappeared was observed at the first steam with one dark silkworm came out of my mouth.	
(G)	I can't sleep, eat and comes with headache. The left check swollen. The hole of the premolar tooth was small but the pain inside was very severe. After two days of mouth steam. I forgot my inconvenient experiences.	

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Medicinal plants are communicated from ancient to recent through careful observation and beliefs. Plant knowledge is sometimes inherited through family lineage. As such, observation and repeated trials become necessary before laboratory/ clinical evaluation especially for commonly used plant in a particular area. The observation and confession obtained from *Xylopia aethiopica pods* and *Amaranthus spinosus* extracts for silkworm removal in individual patients has called for more scientific research clinical investigation. It was observed that the believe of all evil punishment on man when they are in pain has a solution from herbs. Tooth ache is one of the illnesses or sicknesses that could make one believe such an evil act due to un-resisted pain. The independent respondent has swollen check or jaw in common with different intensity of pains as revealed in **Table 4**. Generally, pain endurance is an independent factor in different body function abilities both psychological and physiological. Only one exceptional case in (a) showed sucked out blood and all the others have no report. The silkworm removal in any case showed proportionality to discomfort and pain relief. Others without the worm's removal and still had a serious relief indicated a possibly infection (bacterial) or inflammatory in the pulpitis. Such inflammatory conditions make independent respondent inability to eat and drink as in (A, C. E and G). The ability to sleep is dependent on pain relief as the respondents got a good sleep after the mouth steam. The coming out of the silkworm and sudden painkiller could be responded by the characteristic nature of the phyto-constituents in the combined extract. Alkaloids and phenols (Albuquerque et al., 2020) are well known for such property as pain reliever and anti-inflammatory (Heinrich et al., 2021). Each patient's pain relievers range for one to two days only. In addition to the mouth steaming, the extracts are further diluted before used as warm mouth wash. It was observed that the extract causes mouth irritation if not diluted. This indicates why the evaluation procedure is mainly done through steam method not mouth gurgle. The closer to the mouth for the container used for steaming, the more the diffusion effectiveness on the holes and surrounding gums of the teeth as shown in **Figure 2**. As such, non-variable in terms of colour and age is insignificant in this experiment.

Conclusions

The extracts of *Amaranthus spinosus and Xylopia aethiopica* using fermented corn water showed potential pharmacological benefits for silkworm removal and relieves tooth ache. There is need for further research to be conducted verifying clinical phytochemicals potentials and their synergy. Major chemical compounds responsible for its potency should be isolated and characterized for safety and efficacy in continues used by humans.

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Author Contributions: Yakubu Rufai did the Conceptualization & Investigation. **Sheik, Fatimah**

.A. did the Formal Analysis, Methodology, and Validation, **Abdulmumin, Onize Halimat & Sheik, Fatimah .A.** support Funding Acquisition, Resources, and Project Administration. **Yakubu Rufai** did the Writing of the original Draft, Writing-Review & Edit.

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