

The Synergistic Effect of *Garcinia Kola* on Some Pathogenic Bacteria And Fungi

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Abstract: Due to increasing rate of antimicrobial drug resistance in recent years, this brought about research on use of natural plant for herbal medicine, by the use of *Garcinia kola* pericarp and the seed extract. The ethanolic extract shows a significant inhibition of growth against the tested bacteria (*Klebsiella pneumoniae* 13.00mm, *Escherichia coli* 10.00mm, *Staphylococcus aureus* 10.00mm and *Pseudomonas aeruginosa* 10.00mm). The aqueous extract of both the seed and pericarp has no inhibiting action on all the bacteria and the fungi isolates (0.00mm). The pericarp has the highest zone of inhibition 17.00mm against the tested bacteria compared to the seed extract 4.00mm. The proximate analysis revealed that *Bere* seed (BS) has the highest percentage moisture content (13.05%), *Oje* pericarp (OP) has the highest protein content (11.37%), crude fibre (14.56%) and ash content (7.11%), *Bere* seed (BS) has the highest carbohydrate content (73.19%) and *Oje* seed with the highest crude fat (4.27%). *Bere* pericarp extract (BPE) contain alkaloids, saponin and phenol in an appreciable amount (+++). *Oje* pericarp extract (OPE) has the highest qualitative composition present in appreciable amount (+++) which are (alkaloids, tannin, saponin, phenol and glycosides) likewise phlobatannin in BSE sample. The investigation demonstrated that plant has antimicrobial and greater inhibitory effect which makes it useful in folk and African traditional medicine for treatment of various ailments. The proximate composition reveals that *Bere* seed has the highest percentage of moisture content (13.05%), OP highest protein composition 11.37%, ash composition (7.11%) and crude fibre (14.56%), there was variation in the proximate composition.

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Keywords: antimicrobial, proximate analysis, pericarp, inhibition, ash content and moisture

Introduction

The uses of plants in traditional medicine have a long drawn history, and remain the mainstay of primary health care in most of the third world. Traditional medicine is used by about 60% of the world population, in both developing and is predominantly used (Mythilpyriya *et al.*, 2007). By estimation 60-80% of Africa population depends solely on herbal remedies for its primary health care needs. According to the World Health organization (WHO) up to 80% of the population in Africa depends on traditional herbal medicine for primary health care, accounting for about 20% of the overall drug market (Iwu *et al.*, 2000). Of recent plant materials continue to play a major role in primary healthcare as therapeutic remedies in many developing countries (Jonathan *et al.*, 2005; Jonathan *et al.*, 2007). *Garcinia kola* commonly called "Orogho" in Yoruba language is valued because of its edible nut, the plant exhibits very potent pharmacological activities such as antioxidants, antibacterial, antiviral, antifungal and anti-inflammatory properties. The anti-oxidant property of *Garcinia kola* is attributed to its very high content of ascorbic acid. Phytochemistry of *Garcinia kola* has shown its content to include: benzophenones, xanthenes, biflavonoids, alkaloids, phenols, tannins and saponins. The development and wide spread of resistance of microorganisms to existing antibiotics call for increased efforts in the development of

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antibiotics for treatment of microbial infections and diseases. Although there is a wide range of antibiotics for the treatment of these infection and diseases, the development of resistance to chemotherapeutic agents are increasingly becoming a serious and global problem (Akpulu *et al.*, 1994).

Some of the phytochemicals compounds that have been isolated from *Garcinia kola* include: Oleoresin, tannin, saponins, alkaloids and cardiac glycoside. Others include bi-flavonoids such as kolaflavonone, and 2 hydroxyflavonoids. In addition two new chromanols; garcioic and garcinia together with tocotrienol have been reportedly isolated from bitter kola (Akpulu *et al.*, 1994). Their astringents property makes them useful in preventing diarrhea and controlling hemorrhage due to their ability to precipitate proteins, mucus and constrict blood vessels (Kokwaro, 2009). Saponin shows the potential of the plants to be used to produce mild detergents and intracellular histochemistry staining to allow antibody access to intercellular proteins (Maobe *et al.*, 2013). Flavonoids are used as antioxidants because of their ability to scavenge free radicals such as peroxide and hydroperoxide of lip hydroxyl hence inhibitory oxidation which leads to degenerative diseases (Samatha *et al.*, 2012). It prevents synthesis of flavours that are caused by fat oxidation.

Garcinia kola is used in many tropical countries to fight infectious diseases such as Aids and Ebola Virus; it has shown to possess anti-inflammatory

and antiviral properties (Akinnibosun and Iredije, 2013).

Materials and Method

Garcinia kola nuts was purchased from Bodija and Oje market all in Ibadan, Oyo State, Nigeria, aseptically in an iced pack clean polythene bag, properly labeled and transferred to the Biology Department Laboratory of the Polytechnic Ibadan. Likewise the organisms were collected from Microbiology Department laboratory of The Polytechnic, Ibadan and the samples were identified. The identity of the organisms were confirmed using cultural, morphological and biochemical test as previously described by Olutiola et al.(2000) and they were maintained on agar slant at 4 °C till further use.

Sample Preparation

The pericarp was separated from the seed and was separately oven dried at 45°C for 24h, grinded into powdered form under aseptic condition. Aqueous and absolute Ethanol (98%) was used for the extraction of active compounds from the plant materials. 15g of each samples was suspended in 100 ml of absolute ethanol in 250 ml flask. The same was repeated for the aqueous, vigorously shaken for 30 min and allowed to stand for 48h to effect proper extraction of active ingredient. The suspension was filtered with Whatman filter paper (no1) to obtain the supernatant while the deposit was discarded. The extract after distillation was analysed according to Harbone(1973). The viability of the isolate was determined by sub-culturing the bacteria on Nutrient agar and the fungi on Potato Dextrose agar and the pure cultures obtained were stored on agar slants at 4°C as stock cultures in the refrigerator till further use.

Antimicrobial Sensitivity Test

Effect of Aqueous and Ethanoic Extract on Bacteria Isolate

The agar well diffusion technique was used to determine the antibacterial activity of the plant extracts on various pathogenic bacteria as described by Adegoke and Adebayo-tayo (2009). The extracts were tested against four different pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella species*).

The Mueller Hinton agar was allowed to set in Petri dish plate. The organism was inoculated onto the agar plate by swabbing uniformly on the surface of the plate. One mm cork borer was used to make a hole on the agar plates; 0.5ml of the extract (*Garcinia kola* seed and pericarp in aqueous and ethanol form) was introduced into the hole, the control (aqueous and ethanol) was also prepared too and incubated for 24h at 37°C, the zones of inhibition was measured using a ruler and the results were recorded.

Effect of Aqueous and Ethanoic Extract on Fungi Isolate

The agar diffusion method was used according to method of Akinnibosun and Rehoboth(2015). A fresh cultures of 0.5ml of different pathogenic fungi (*Aspergillus niger*, *Aspergillus terreus*, *Candida albicans*, *Rhizopus oryzae*) was inoculated into separate sterile Petri dish plate with each organism containing the seed and pericarp from two different markets, the sterile media was aseptically poured into each Petri dish, gently rocked for proper mixture and the agar was allowed to solidify. Afterwards, two wells were bored with sterilize cork borer of 1mm diameter on each plate (ethanol extract of the seed and ethanol extract of the pericarp) 0.5ml of each of the ethanol extract of the samples was introduced into the well. The plates were allowed to stand for one hour for proper diffusion and it was later incubated at 37°C for 72h. The sensitivity of the tested organisms on the aqueous and ethanol extract was checked for zone of inhibition around the wells. The diameter of the zone of inhibition was measured to the nearest millimeter (Akinnibosun and Rehoboth, 2015).

Phytochemical Screening

Phytochemical screening (alkaloid, tannin, phlobatannin, saponin, steroids, flavonoids, anthraquinones, terpenes, cardenolides, phenol, chalcones and cardiac glycoside) were carried out on the powder form of the pericarp and seed extracts from the two different markets using standard procedure to identify the constituents according to the method of Sofowora (1982).

Proximate Analysis

The Proximate composition was carried out on *Garcinia kola* seed and pericarp to determine its nutritional composition based on the following parameters (crude protein, crude fat, crude Fibre, ash content, moisture content and carbohydrate) according to method of A.O.A.C.(2010).

Results and Discussion

Evaluation of the antimicrobial properties and synergistic effect of *Garcinia Kola* on the pathogenic bacteria isolates was expressed as a measure of the diameter of the inhibition of growth in millimeters. The antimicrobial activity of ethanol bitter kola extraction and aqueous bitter kola extraction of the pathogenic bacteria shows that ethanol extract had antimicrobial properties which prevent the growth of the tested organisms. The study on *Garcinia Kola* samples from different market shows that the pericarp as a higher zone of inhibition against the pathogenic bacteria as shown in Table 1. Of all the extract screened, ethanol extract had a higher antimicrobial activity against all the tested pathogenic organisms (*Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli*) than the aqueous

extract which is in agreement with Fabeku (2006) who also reported that bitter kola has strong antibiotic activities and it is found to be very effective against Gram negative(-ve) and Gram positive (+ve) bacteria. They further stated that *Garcinia kola* inhibit growth of pathogenic organisms. *Oje* pericarp extract (OPE) was most active against *Klebsiella pneumonia* zone of inhibition (13mm, 17mm and 17mm) after (24, 48 and 72 h) and least active against *Pseudomonas aeruginosa* with zone of inhibition (8mm) after 24h. This result was similar to the work of Adegboye et al. (2008) who reported that ethanol extract of *Garcinia kola* exhibited antimicrobial activities *in-vitro* against both Gram positive (-ve) and Gram negative(+ve) bacteria. *Oje* pericarp extract has a higher zone of inhibition than BPE; this could be as a result of exposure of bitter kola to the environment, presence of chemical compounds in the pericarp and the differences in the antimicrobial properties of the pericarp. This difference may also be due to the age of the pericarp and seed, freshness, physical factors (temperature, light and water), attack by microbes in the environment, adulteration and substitution of the *Garcinia kola* dosage. In this study, the length of incubation had a change on the antimicrobial activity. The zone of inhibition increases as the length of incubation period increases on some of the pathogenic bacteria. In addition the pericarp extract has a wider zone of inhibition than the seed. The aqueous extraction shown in Table 2 shows no zone of inhibition against all the tested bacteria, this could be as a result of absence of bioactive compounds that is present in ethanol which is not available in water. It could also be as a result of therapeutic agent in the plant. The spectrum of activity shows that both ethanol extract shown in Table 3 and aqueous extract illustrated in Table 4 does not inhibit the pathogenic fungi, this could be as a result of pathogenicity of the organisms or

the toxins in them. The result further showed that ethanol extract inhibit pathogenic bacteria compared to the aqueous, while ethanol and aqueous extraction of both pericarp and seed does not inhibit all the tested pathogenic fungi organisms which is in agreement with Iwu et al. (1990) who reported that *Garcinia kola* contains some phytochemical component present in antibiotics. Table 5 Show the phytochemical composition, the qualitative composition of *Garcinia kola* seed and pericarp from two different markets. The composition of the pericarp and seed indicated that *Berepericarp* extract contains alkaloids, saponin and phenol in an appreciable amount (+++) which is similar to the work of Jackie et al.(2014) who reported the presences of tannins, saponins, flavonoids, terpenoids, glycosides and alkaloids with the absence of steroids and phenols. The *Berepericarp* extract has in abundance some anti-nutrient in an appreciable amount than *Bere* seed extract (alkaloid, saponin and phenol). *Ojepericarp* extract has the highest qualitative composition of alkaloids, tannin, saponin, phenol and cardiac glycoside which are present in an appreciable amount (+++) while in moderate amount in *Oje* seed. The qualitative and quantitative composition of *Garcinia kola* contain some anti-nutrient compounds in abundance such as, alkaloids, tannin, cardenolide, phenol, chalcones, phlobatannin, saponin, flavonoids, anthraquinones, steroids, terpenes, cardiac glycosides. The composition of the pericarp and the seed indicated that the *Berepericarp* extract contains alkaloids, saponin and phenol which are present in an appreciable amount (+++), tannin, anthraquinones and cardiac glycosides are present in a moderate amount (++), phlobatannin, flavonoids, steroids, terpenes and cardenolides are present in a minute amount (+) while chalcones was completely absent (-).

Table 1: Shows the antimicrobial activity of *Garcinia kola* seed and pericarp ethanoic extract against some pathogenic bacteria.

Key: OSE= *Oje* seed extract, OPE= *Oje* pericarp extract, BSE= *Bere* seed extract

Organisms	Sample	Zone of inhibition (mm)	
		Incubation time (h)	
		24	48/72
<i>Klebsiella pneumonia</i>	OSE	3.00	3.00
	BSE	4.00	4.00
	OPE	13.00	17.00
<i>Pseudomonas aeruginosa</i>	BPE	10.00	14.00
	OSE	4.00	5.00
	BSE	4.00	4.00
<i>Escherichia coli</i>	OPE	8.00	10.00
	BPE	7.00	9.00
	OSE	3.00	3.00
<i>Staphylococcus aureus</i>	BSE	3.00	3.00
	OPE	11.00	13.00
	BPE	10.00	11.00
	OSE	5.00	5.00
	BSE	4.00	4.00
	OPE	9.00	9.00
	BPE	10.00	10.00

BPE= *Bere* pericarp extract

Table 2: Shows the antimicrobial activity of *Garcinia kola* seed and pericarp aqueous extract on some pathogenic bacteria

Organisms	Sample	Zone of inhibition (mm)		
		Incubation time (h)		
		24	48	72
<i>Klebsiella pneumonia</i>	OSE	0.00	0.00	0.00
	BSE	0.00	0.00	0.00
	OPE	0.00	0.00	0.00
	BPE	0.00	0.00	0.00
<i>Pseudomonas aeruginosa</i>	OSE	0.00	0.00	0.00
	BSE	0.00	0.00	0.00
	OPE	0.00	0.00	0.00
	BPE	0.00	0.00	0.00
<i>Escherichia coli</i>	OSE	0.00	0.00	0.00
	BSE	0.00	0.00	0.00
	OPE	0.00	0.00	0.00
	BPE	0.00	0.00	0.00
<i>Staphylococcus aureus</i>	OSE	0.00	0.00	0.00
	BSE	0.00	0.00	0.00
	OPE	0.00	0.00	0.00
	BPE	0.00	0.00	0.00

Key: OSE= *Oje* seed extract, OPE= *Oje* pericarp extract, BSE= *Bere* seed extract
BPE= *Bere* pericarp extract

Table 3: Antimicrobial activity of ethanoic extract on pathogenic Fungi

Organisms	BSE	BPE	OSE	OPE
<i>Aspergillus niger</i>	0.00	0.00	0.00	0.00
<i>Rhizopus oryzae</i>	0.00	0.00	0.00	0.00
<i>Aspergillus terreus</i>	0.00	0.00	0.00	0.00
<i>Candida albican</i>	0.00	0.00	0.00	0.00

Key: OSE= *Oje* seed extract, OPE= *Oje* pericarp extract BSE= *Bere* seed extract, BPE= *Bere* pericarp extract

Table 4: Antimicrobial activity of aqueous extract on pathogenic fungi

Organisms	BSE	BPE	OSE	OPE
<i>Aspergillus niger</i>	0.00	0.00	0.00	0.00
<i>Rhizopus oryzae</i>	0.00	0.00	0.00	0.00
<i>Aspergillus terreus</i>	0.00	0.00	0.00	0.00
<i>Candida albican</i>	0.00	0.00	0.00	0.00

Key: OSE= *Oje* seed extract, OPE= *Oje* pericarp extract BSE= *Bere* seed extract
BPE= *Bere* pericarp extract

Bere seed extract contains phenol which was the only one present in an appreciable amount (+++), alkaloids, saponin, steroids, terpenes are present in a moderate amount (++), tannin, flavonoids, anthraquinones and cardiac glycosides are present in a minute amount (+)

while chalcones is completely absent (-). *Oje* pericarp extract results contains alkaloids, tannin, saponin, phenol and cardiac glycosides are present in an appreciable amount (+++), phlobatannin, flavonoids, anthraquinones, steroids, terpenes, cardenolides are

present in a moderate amount (++) while chalcones is completely absent (-). *Ojeseed* extract contains alkaloids and phenol present in an appreciable amount (+++), tannin, saponin, anthraquinones, steroids, terpenes are present in a moderate amount (++), phlobatannin, flavonoids, cardiac glycosides are present in a minute amount (+), while chalcones and cardenolides are completely absent. This result shows that *Oje* seed extract has a higher bioactive compounds present in an appreciable amount (+++) and moderate amount (++) than *Bere* pericarp extract and *Ojeseed* extract which has more appreciable amount (+++) of anti-nutritional composition than *Bere* seed extract. The quantitative phytochemical screening in Table 6 shows that only phenol has the highest value in *Bere* seed extract (0.2480), terpenes with the lowest value (0.0002) and chalcones with no value (0.0000). In *Bere* pericarp extract saponin has the highest value, cardenolide with the lowest (0.0004), and chalcones with no value (0.000). In *Oje* seed extract, terpenes have the lowest value (0.0004), phenol with the highest value (0.259), cardenolide and chalcones with no value (0.0000). In *Oje* pericarp extract saponin has the highest value (0.2250), cardenolides with the lowest (0.0007) and chalcones with no value (0.000). It was discovered that phenol was present in high amount in both the seed extract and saponin has highest amount in both the pericarp extract. Phytochemicals are biologically active compounds, found in plants in small amount which contribute significantly to protection against degenerative diseases which is in agreement with Dreosti *et al.* (2000) who reported phytochemicals significance in the treatment of disease causing infection. Flavonoids have protective effect including anti-inflammatory, anti-oxidant, antiviral and anticarcinogenic properties. *Garcinia kola* is a good dietary source of flavonoids which is in agreement with the report of Middleton *et al.* (2000) who reported the presence of flavonoids in *Garcinia kola*. Proximate analysis is a conventional system that gives the nutrient

present in a particular food samples. The gross nutrient discovered, in Table 7 are moisture content, ash content, crude protein, crude fat, crude fibre and carbohydrate which is in agreement with Onyeike and Osuji (2003) who reported that individual nutrient are present in *Garcinia kola*. Carbohydrates are the most abundant biological molecules which play important role in the body as sources of energy was in support of Voet (2008) who reported that carbohydrate contains three elements namely, carbon, nitrogen and oxygen which supplies energy to the body. Carbohydrate composition of the pericarp and the seed extract from two different market showed that both the seed from the different markets has high carbohydrate content compared to the pericarp, the *Bere* seed extract has the highest carbohydrate content (71.5%). Protein, another class of food often times referred to as the nitrogen containing natural products has been proved to be essential for the survival of human being and animals which is in support with Voet (2008) who discovered that protein contain building blocks called amino acids. The pericarp serves as a source of protein compared to the seed. The *Bere* seed extract (13.05 %) has the highest moisture contents, *Ojeseed* extract (12.43%), *Bere* pericarp (8.37%) and *Oje* pericarp (6.95%). It shows that both seed has higher level of moisture than the pericarp and this pericarp serves as protective for the seed against microorganism attack. The fat composition in this study shows that the fat contents ranges from 2.25 (*Bere* pericarp) to 4.27% (*Ojeseed*). Both seeds has a high level of fat which when consumed can supply fat to the body. It was discovered that the crude fibre contents of *Oje* pericarp (14.56%) has the highest composition followed by *Bere* pericarp (13.38%), *Oje* seed (1.48%) and *Bere* seed (1.35%). The ash content shows that the pericarp has a high composition of ash than the seed which ranges from *Oje* pericarp (7.11%), *Bere* pericarp (6.89%), *Oje* seed (3.53%), *Bere* seed (3.15%).

Table 5: Shows phytochemical composition of *Garcinia kola* seed and pericarp extract from various markets.

PHYTOCHEMICALS %	<i>Garcinia kola</i> extract			
	Seed		Pericarp	
	OSEBSE	OPEBPE		
Alkaloid	+++	++	+++	+++
Tannin	++	+	+++	++
Phlobatannin	+	-	++	+
Saponin	++	++	+++	+++
Flavonoids	+	+	++	+
Anthraquinones	++	+	++	+
Steroids	++	++	++	+
Terpenes	++	++	++	+
Cardenolides	-	-	++	+

Phenol	+++	+++	+++	+++
Chalcones	-	-	-	-
Cardiac glycoside	+	+	+++	++

Key: OSE= *Oje* seed extract, BSE= *Bere* seed extract, OPE= *Oje* pericarp extract BPE= *Bere* pericarp extract

+++ = Present in an appreciable amount

++ = Present in a moderate amount

+ = Present in a minute amount

- = Completely absent

Table 6: Shows the quantitative analysis of *Garcinia kola* seed and pericarp extract from various markets.

PHYTOCHEMICALS %	<i>Garcinia kola</i> extract			
	OSE	BSE	PericarpOPE	BPE
Alkaloid	0.0229	0.0230	0.1340	0.1290
Tannin	0.0470	0.0440	0.0250	0.0210
Phlobatannin	0.0280	0.0250	0.0090	0.0120
Saponin	0.1870	0.1830	0.2250	0.2170
Flavonoids	0.0041	0.0036	0.0026	0.0031
Anthraquinones	0.0037	0.0031	0.0018	0.0014
Steroids	0.0011	0.0008	0.0023	0.0010
Terpenes	0.0004	0.0002	0.0015	0.0009
Cardenolides	0.0000	0.0000	0.0007	0.0004
Phenol	0.2590	0.2480	0.1280	0.1270
Chalcones	0.0000	0.0000	0.0000	0.0000
Cardiac glycoside	0.0123	0.1160	0.1140	0.1140

Key: OSE= *Oje* seed extract, BSE= *Bere* seed extract, OPE= *Oje* pericarp extract BPE= *Bere* pericarp extract

Table 7: Proximate composition analysis of *Garcinia kola* seed and pericarp extract from various markets.

SAMPLE	Percentage of proximate composition (%)					
	CP	CFA	CFR	A	M	CHO
<i>Bere</i> seed extract	5.37	3.89	1.35	3.15	13.05	73.19
<i>Bere</i> pericarp extract	9.25	2.25	13.38	6.89	8.37	59.86
<i>Oje</i> seed extract	6.79	4.27	1.48	3.53	12.43	71.5
<i>Oje</i> pericarp extract	11.37	2.78	14.56	7.11	6.95	57.23

Key: CP = Crude protein, CFA = Crude fats, CFR = Crude fibre, CA = Crude ash, M = Moisture,

CHO = Carbohydrate

Conclusion

The excellent performance of the ethanol solute extract of *Garcinia kola* against the tested pathogenic bacteria and fungi organisms suggested frequent input of *Garcinia kola* in food and pharmaceutical product since ethanol was seen as a best solvent for the extraction compared to aqueous. This therefore implies that effort should be made by pharmaceutical companies in carrying out more research work on bitter kola in the development of new

drugs, which contains more biochemical active compounds. There is also need to investigate the antimicrobial potency of the pericarp against wider range of pathogenic clinical isolates in order to obtain a more accurate evaluation of the plant therapeutic potential. Furthermore, it will be necessary to elucidate the mechanism of action, as well as their level of side effects when it is been consumed in a higher amount. The demonstration of antimicrobial activity against gram positive (-ve) and gram negative (+ve) bacteria is

an indication that the plants are potential source for production of drugs with broad spectrum of activities. Most especially the pericarp which are seen as a waste product by people during the consumption of the seed showed to be more beneficial than the seed base on this study. It can be deduced that pericarp has a strong therapeutic properties compared to the seed. Furthermore, base on the nutritional screening *Garcinia kola* seed and pericarp can be used as a good source of carbohydrate, protein and also a good source of minerals necessary for metabolic activities in the body. The phytochemical composition also shows its usefulness in medical sciences and supplement against diseases. It has been established through the nutritional screening, phytochemical and antimicrobial activities that *Garcinia kola* pericarp can be consumed together with the seed in treatment of diseases and therefore further work should be carry out to determine the synergistic effects of *Garcinia kola* on other pathogenic fungi and bacteria isolates and also their effectiveness in order to decrease death rate of people who cannot afford to buy expensive antibiotics to treat themselves.

Recommendation

This study has revealed the use of pericarp and seed extract of *Garcinia kola* as a substitute of antibiotics. Indiscriminate use of antibiotics has led to the emergence of drug resistant strains which has a significant impact on the patient's mortality.

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