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(RESEARCH ARTICLE)



Determination of bioactive constituents of *Spondias mombin* leaves by GC-MS analysis

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Abstract

The therapeutic capabilities of abundant herbal products have been known. However, further researches are required to properly screen, isolate and probably characterize their various active components. In this study, an attempt was made to identify the compounds present in the ethanol and n-hexane extracts of *S. mombin* leaves using gas chromatography and mass spectrometry (GC-MS) technique. *Spondias mombin* leaves were collected, air dried and pulverized into powdery form and extracted with ethanol and n-hexane at room temperature. The ethanol and n-hexane extracts of *S. mombin* leaves were analyzed by GC-MS. The GC-MS analysis of the ethanol extract of *S. mombin* leaves showed twenty-five (25) compounds, while twenty-nine (29) compounds were identified in n-hexane extract of *S. mombin* leaves. However, both extracts had ten compounds in common: n-Hexadecanoic acids, Phytol, Phenol 3-pentadecyl-, Phenol 3-methyl-, Phenol 2-methyl-, 2H-1-Benzopyran-6-ol, Gamma-Tocopherol, Vitamin E, Lup-20(29)-en-3-one and Stigmast-4-en-3-one. The percentage proportion of these compounds differed in each extract. In view of this, the possible biological properties of these identified compounds suggested that *S. mombin* might possibly be potential therapeutic agents.

Keywords: GC-MS; Spondias mombin; Extract; Therapeutic agents.

1. Introduction

Traditional medicine has remained a great alternative basis of medicine worldwide, particularly in low income countries. It has been stated that 80 % of the world populace (about 4 billion people) are dependent mainly on herbal therapies and traditional therapeutic practice for their well-being care (Ekor, 2014). The increasing occurrence of resistance (particularly to antibiotics), detrimental side effects, cost of accessible therapies and the well understanding of illness conditions has led to a renewed curiosity in the development of newer molecules from natural bases (Cowan, 1999). Therefore, aggressive screening of flora used in traditional medicine as well as an attempt to recognize and undertake chemical clarification of the compounds accountable for their stated activities are been undertaken by researchers (Elufioye and Berida, 2018).

Gas Chromatography Mass Spectrometry (GC-MS) is one of the compatible techniques advantageous in recognizing the bioactive components of long chain, alcohols, branched chain hydrocarbons, esters, acids, etc present in crude plant materials (Sathiyabalan *et al.*, 2014). Gas chromatography (GC) is broadly used for compound analysis, drug screening and assessment resulting from environmental pollution. Mass spectrometry (MS) measures the mass-to-charge ratio of ions of analysts and is a plot of strength as a function of mass-to-charge ratio (Kim *et al.*, 2005; Shah *et al.*, 2019). Relying on the database of National Institute of Standards and Technology Chemistry Web Book (2019) for diverse compounds, researchers can thus recognize and quantify these potential compounds and components.

Amongst numerous known therapeutic plants is *Spondias mombin. Spondias mombin L.* (Family-Anacardiaceae) also recognized as Hog plum, is a plant that grows in nearly every part of the world. It is a deciduous tree that produces many

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fruits which is about 20 m tall and it grows in the rain forest and the coastal part of Africa. It is recognized locally as "iyawe" and "iyeye" by the Hausa and Yoruba people of Nigeria respectively (Dharmananda, 2003). Tradomedicine physicians across Africa use all parts of the plant for therapeutic purposes. The fruits' decoction is drunk as a febrifuge and diuretic, while the decoction of the leaves and stem-bark are used as an emetic, dysentery and anti-diarrhea (Osuntokun *et al.*, 2018). *Spondias mombin* is a good recipe for the treatment of gonorrhea and leucorrhea as well as for haemorrhoids (Li *et al.*, 2003). A tea of the leaves and flowers of *S. mombin* is taken to relieve stomach ache, biliousness, cystitis, urethritis, throat and eye inflammations. Herbalists in Southwest Nigeria use the plant in the treatment of diabetics, psychiatric disorders, typhoid, nervous disorders and tuberculosis (Newman *et al.*, 2008). The extract of the powder of the dried leaves and fresh crushed leaves are used for healing inflammation, wounds, varicose ulcers, burn and frost-bite in herbal medicine (Shaarawy *et al.*, 2009). The bark, fruits and leaves of *S. mombin* are said to be rich in antioxidants and other valuable phytochemicals (Igwe *et al.*, 2011; Omoregie and Oikeh, 2015). However, much work has not been done on the identification of the compounds accountable for these stated activities. Using GC-MS, we aimed to identify the compounds present in the ethanol and n-hexane extracts of *S. mombin* leaves.

2. Materials and methods

2.1. Collection of Plant Sample

Spondias mombin leaves were obtained from the Faculty of Pharmacy's premises, University of Benin, Benin City, Edo State, Nigeria and authenticated at the Department of Plant Biology and Biotechnology with voucher number UBH-S345. The plant was air dried under room temperature and pulverized into powdery form which was kept in an air-tight container ready for the analysis.

2.2. Plant extraction

Cold extraction method was used in this study, by dissolving the pulverized plant material in ethanol and n-hexane as solvents. The filtrate for each extraction was combined, and evaporated to dryness at room temperature.

2.3. Gas Chromatography - Mass Spectrometry (GC-MS)

GC-MS analysis of the ethanol and n-hexane extracts of *S. mombin* leaves were performed using Hewlett-Packard (HP) 6890 series (Agilent) Gas Chromatography System, interfaced to HP 5973 series (Agilent) mass spectrometer, equipped with an autosampler and a single capillary injector. TR-FAME (Thermo 260 M142P) (70 % cyanopropylpolysilphenylenesiloxane) capillary GC column (30 m x 0.25 mm, i.d., x 0.25 μ m film thickness) was also used. This was done by dissolving the sample in the organic solvent till it dissolved completely. GC condition was maintained at 100 – 280 °C as 5 °C/min. 2 μ l of sample was injected into the column. The helium gas was allowed to move at 1 ml/min through the column and the compound split in the ratio of 1:10. After the program, mass spectrometer scanned the compounds separated and each peak area was measured to find the compounds present at the area. The results were represented as chromatogram graph in the GC-MS.

3. Results

The GC analysis and mass spectral data of the ethanol and n-hexane extracts of *S. mombin* leaves showing compound name, retention time, peak area (%), molecular weight (g/mol), structural formula, compound nature and structure of compound is presented in Tables 1 and 2. Twenty five (25) compounds were identified in the ethanol extract (Table 1), while twenty-nine (29) compounds were detected in the n-hexane extract of the *S. mombin* leaves (Table 2). Figures 1 and 2 depict the chromatograms of ethanol and n-hexane extracts of *S. mombin* leaves respectively with their peak areas. In ethanol extract, phenol, 2-methyl- gave the highest peak at 21.918 retention time (Figure 1) while phenol, 3-methyl-gave the highest peak at 21.941 retention time in n-hexane extract (Figure 2).

| S/N | NC | RT | A (%) | MW (g/mol) | SF | CN | SC |
|-----|-----------------------------|--------|-------|------------|--|------------------------------|--|
| | 2(4H)-Benzofuranone | 9.158 | 1.06 | 166.2170 | C ₁₀ H ₁₄ O ₂ | Terpene | |
| | Bicyclo [3.1.1] heptane | 10.228 | 1.41 | 138.2499 | C ₁₀ H ₁₈ | Bicyclic Monoterpene | The second secon |
| | n-Hexadecanoic acid | 11.441 | 1.48 | 256.4241 | $C_{16}H_{32}O_2$ | Palmitic acid | |
| | | | | | | | н |
| | Hexadecanoic acid | 11.818 | 0.88 | 284 | $C_{18}H_{36}O_2$ | Ethyl ester | |
| | 2-Hydroxy-1,8-naphthyridine | 12.402 | 0.37 | 162.148 | C8H6N2O2 | Quinoxalinedione | |
| | Phytol | 13.444 | 2.61 | 296.539 | C ₂₀ H ₄₀ O | Acyclic diterpene alcohol | H.O. H. |

Table 1 Compounds Identified in Ethanol Extract of Spondias mombin Leaves by Gas Chromatography Mass Spectrometry (GC-MS) Method

| 7, 10, 13-Hexadecatrienoic acid | 13.827 | 1.03 | | | | |
|---|--------|-------|------------|-----------------------------------|----------------------------------|-----------------|
| Diisooctyl phthalate | 20.075 | 0.61 | | | | |
| Phenol, 3-methyl- | 21.803 | 8.74 | 108.1378 | C ₇ H ₈ O | Methylphenols | |
| | 21.010 | 24.25 | 100 1270 | | | СН |
| Phenol, 2-methyl- | 21.918 | 21.25 | 108.1378 | C7H8O | Metnyiphenois | |
| | | | | | | Ж _{он} |
| Phenol, 3-pentadecyl- | 22.273 | 5.93 | 304.5099 | C ₂₁ H ₃₆ O | Alkylresorcinol(Phe | H0 |
| | | | | | | |
| Silane, diethyl (2-bromo-4-fluoroph enoxy) undecyloxy- | 24.372 | 1.22 | 447.497243 | C21H36BrFO2Si | | |
| Silane, diethylpentadecyloxy (1- phenylpropoxy)- | 24.670 | 1.80 | | | | |
| Phenol, 4-(2-aminoethyl)- | 24.784 | 1.00 | | | | |
| 2H-1-Benzopyran-6-ol | 25.563 | 2.46 | 402.6529 | C27H46O2 | Vitamin E (Delta- Tocopherol) | |
| gammaTocopherol | 26.936 | 0.76 | 416.69 | $C_{28}H_{48}O_2$ | Vitamin Ε (γ- Tocopherol) | |
| | | | | | | |

| Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15-hexadecamethyl | 27.840 | 0.54 | | | | |
|--|--------|-------|--------------------|--|----------------------------------|--------|
| Vitamin E | 27.966 | 1.62 | 430.717 | $C_{29}H_{50}O_2$ | Vitamin E (alpha- tocopherol) | |
| 5-Methyl-2-phenylindolizine | 28.916 | 1.93 | 207.276 | C ₁₅ H ₁₃ N | | |
| .gammaSitosterol | 30.106 | 16.16 | 414.718 414.718 | C ₂₉ H ₅₀ O C ₂₉ H ₅₀ O | Triterpe noid phytosterol | |
| Lup-20(29)-en-3-one | 30.810 | 7.14 | 424.713 | $C_{30}H_{48}O$ | Triterpene | |
| Alpha-Amyrin | 31.124 | 7.07 | 426.729 | C ₃₀ H ₅₀ O | Triterpene | -tptpt |



Key: NC=Name of compound, RT= Retention time, A (%) = Peak Area (%), MW (g/mol) = Molecular weight (g/mol), SF= Structural formula, CN= Compound nature, SC= Structure of compound.

| S/N | NC | RT | A (%) | MW (g/mol) | SF | CN | SC |
|-----|------------------------------------|--------|----------|------------|--|-----------------------------|----|
| | Decanoic acid, ethyl ester | 5.170 | 1.75 | 200.3178 | C ₁₂ H ₂₄ O ₂ | Fatty acid (ester) | |
| | Dodecanoic acid, ethyl ester | 7.556 | 3.15 | 228.3709 | C14H28O2 | Fatty acid (ethyl ester) | |
| | Tetradecanoic acid, ethyl ester | 9.747 | 2.60 | 256.4241 | $C_{16}H_{32}O_2$ | Fatty acid | |
| | 1,2- Dihexylcyclopropene | 10.234 | 0.34 | | | Cycloalkane | |

Table 2 Compounds Identified in N-Hexane Extract of Spondias mombin Leaves by Gas Chromatography and Mass Spectrometric (GC-MS) Method

| Tetradecanal | 10.268 | 0.45 | 212.3715 | C ₁₄ H ₂₈ O | Myristic acid | |
|--|--------|------|----------|--|----------------------|----------|
| n-Hexadecanoic acid | 11.458 | 0.27 | 256.4241 | C ₁₆ H ₃₂ O ₂ | Saturated fatty acid | CH CH |
| Pentafluoropropionic acid, pentadecyl ester | 11.813 | 0.34 | 374.436 | $C_{18}H_{31}F_5O_2$ | | |
| | | | | | | |
| | | | | | | |
| Phytol | 13.444 | 0.66 | 296.539 | C ₂₀ H ₄₀ O | Diterpene | → |
| 9,12-Octadecadienoic acid (Z,Z) | 13.867 | 8.93 | 280.452 | C ₁₈ H ₃₂ O ₂ | Glycosides | OH CH |
| | | | | | | |









Key: NC=Name of compound, RT= Retention time, A (%) = Peak Area (%), MW (g/mol) = Molecular weight (g/mol), SF= Structural formula, CN= Compound nature, SC= Structure of compound.



Figure 1 Chromatogram of Spondias mombin leaves' ethanol extract by GC-MS method.



Figure 2 Chromatogram of Spondias mombin leaves' n-hexane extract by GC-MS method.

4. Discussion

The determination of phytochemicals in useful and regularly consumed plant products is significant. Thus, using gas chromatography and mass spectrometry (GC-MS) method, this study attempted to identify and quantify compounds present in the ethanol and n-hexane extracts of *S. mombin* leaves. Twenty five (25) compounds were identified in the ethanol extract of *S. mombin* leaves viz: 2(4H)-Benzofuranone (1.06%), Bicyclo [3.1.1] heptane (1.41%), n-Hexadecanoic acid (1.48%), Hexadecanoic acid (0.88%), 2-Hydroxy-1,8-naphthyridine (0.37%), Phytol (2.61%), 7, 10, 13-Hexadecatrienoic acid (1.03%), Phenol, Diisooctyl phthalate (0.61%), Phenol, 3-methyl- (8.74%), Phenol, 2-methyl-

(21.25%), 3-pentadecyl- (5.93%), Silane, diethyl (2-bromo-4-fluoroph enoxy) undecyloxy- (1.22%), Silane, diethylpentadecyloxy (1-phenylpropoxy)- (1.80%), Phenol, 4-(2-aminoethyl)- (1.00%), 2H-1-Benzopyran-6-ol (2.46%), gamma.-Tocopherol (0.76%), Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15 hexadecamethyl (0.54%), Vitamin E (1.62), 5-Methyl-2-phenylindolizine (1.93%), gamma.-Sitosterol (16.16%), Lup-20(29)-en-3-one (7.14%), alpha-Amyrin (7.07%), Stigmast-4-en-3-one (2.73%), Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15-hexadecamethyl- (1.36%), and 1-Dimethyl(phenyl)silyloxypentane (3.89%). Spondias mombin n-hexane leaves extract also revealed twenty-nine compounds. The compounds were Decanoic acid, ethyl ester (1.75 %), Dodecanoic acid, ethyl ester (3.15%), Tetradecanoic acid, ethyl ester (2.60%), 1.2-Dihexylcyclopropene (0.34%), Tetradecanal (0.45%), n-Hexadecanoic acid (0.27%), Pentafluoropropionic acid, pentadecyl ester (0.34%), Phytol (0.66%), 9,12-Octadecadienoic acid (Z,Z) (8.93%), Phenol, 3-pentadecyl- (2.31%), Phthalic acid, di(oct-3-yl) ester (0.53%), Phenol, 2methyl- (6.16%), Phenol, 3-methyl- (17.80%), 3-Amino-4-pyrazolecarbonitrile (1.37%), Z,Z-10,12-Hexadecadien-1-ol acetate (1.63%), Squalene (0.60%), 1-Pyrrolidinebutanoic acid (0.80%), Eicosane (0.58%), 2H-1-Benzopyran-6-ol (1.50%), gamma.-Tocopherol (0.89%), Hexadecane, 1-iodo- (5.81%), Vitamin E (7.82%), Eicosane, 9-octyl- (1.53%), beta.-Sitosterol (8.59%), Lup-20(29)-en-3-one (5.32%), 4-Dehydroxy-N-(4,5-methylenedioxy-2- nitrobenzylidene) tyramine (1.07%), Stigmast-4-en-3-one (2.82%), Silane, dimethyl (2-naphthoxy) heptyloxy- (0.78%), and Cyclotrisiloxane, hexamethyl (0.83%).

These compounds belong to different classes of compounds such as fat soluble vitamin E, steroids, fatty acids, terpenoids, terpenoids, terpenois. These bioactive chemicals produced by plants are used to support well-being and fight against contagions and many of them are sold as herbal medications or foods. Their usage has upsurge over the last era not only because of their low cost, ease of access, but also the belief that natural therapies have fewer harmful effects as related to synthetic analogues (Hadi *et al.*, 2016).

Remarkably, both extracts evaluated had ten compounds in common. These are n-Hexadecanoic acid, Phytol, Phenol 3pentadecyl-, Phenol 3-methyl-, Phenol 2-methyl-, 2H-1-Benzopyran-6-ol, gamma-Tocopherol, Vitamin E, Lup-20(29)en-3-one, and Stigmast-4-en-3-one. These compounds identified have irresistible therapeutic usages. Phytol which was identified in the ethanol and n-hexane extracts of *S. mombin* leaves have been stated advantageous as an antibacterial agent active against *Staphylococcus aureus*, a precursor for Vitamins K and E, effective at different phases of arthritis (Inoue *et al.*, 2005). It's antioxidant, anticancer and antinociceptive activities were reported by Ammal and Bai (2013). The presence of phytol in both extracts was in accordance with the report of Bekinbo *et al.* (2020), who studied methanol extract of *S. mombin* leaves. Ovuakporie-Uvo *et al.* (2018) also identified phytol in methanol extract of *Desplatsia dewevrei* leaves and fruits.

Vitamin E and gamma-Tocopherol identified in this study were also reported in the studies of Guedes *et al.* (2020) in *S. mombin* and *S. tuberose.* Recently, Abraham *et al.* (2019) revealed that vitamin E isoforms had anticancer activity with the exception of non-alpha-Tocopherol form. Gamma Tocopherols had been revealed to prevent the development of different kinds of tumor, including prostate tumor (Huang *et al.*, 2014).

The identification of n-Hexadecanoic acids also known as palmitic acid in both extracts were reported to possess cyclooxygenase and antioxidants properties (Benkendorff *et al.*, 2005; Suhaj, 2006). Perez-Pinzon and Lin (2013) stated that saturated fatty acids or polyunsaturated such as n-Hexadecanoic acids and α -linolenic acid have ability of offering neuroprotection after ischemia. Agreeing to World Health Organization, the intake of n-Hexadecanoic acids upsurges the threat of developing cardiovascular illness (Uauy *et al.*, 2009). Bekinbo *et al.* (2020) and Qureshi *et al.* (2020) also reported the presence of n-Hexadecanoic acids in methanol extract of *S. mombin* leaves and n-hexane extract of *Kochia indica* stem respectively. Elufioye and Berida (2018) also identified n-Hexadecanoic acids in n-hexane extract of the stem-bark and whole fruit of *Spondias purpurea.* n-Hexadecanoic acids were found earlier in *Syzygium cumini* and stated to have antifungal activities (Javaid *et al.*, 2018). Similarly, Bhawanker *et al.* (2013) isolated n-Hexadecanoic acids from ethanol extract of *Aloe vera* and also recognized their antifungal and antibacterial activities against *Candida albicans* and *Streptomyces greseus.* n-Hexadecanoic acids, a significant antimicrobial compound identified in this study was also isolated from *Brassica nigr*a oil and assessed against *Aspergillus niger, Candida albicans, Staphylococcus aureus* and *Pseudomonas aeruginosa* through cup plate agar diffusion assay by Abdel-Karim (2017).

Squalene, one of the compounds identified in n-hexane extract of *S. mombin* leaves in this study was also reported in the crude ethyl acetate root extract of *S. mombin* by Osuntokun *et al.* (2018). Guedes *et al.* (2020) also reported squalene in n-hexane extract from *S. tuberose* and *S. mombin* leaves. It has been reported that squalene acts as a drug transporter by chemically linking with medicines to advance certain physicochemical properties. For instance, administration of squalene-doxorubicin nanohybrids caused higher decrease of pancreatic cancers when related with free doxorubicin (Wicki *et al.*, 2015). Consequently, squalene based nanoparticles have been deliberated to be likely candidates for anticancer medications (Kotelevets *et al.*, 2017; Saha *et al.*, 2015). In the nutritious context, virgin olive oil is a vital basis of

squalene (Beltrán *et al.*, 2016). Intake of olive oil has been interrelated with lower risk of tumor growth in several cancer kinds (Newmark, 1999; Lozano-Grande *et al.*, 2018). Kalvodona (2010) stated that squalene is not very vulnerable to peroxidation and seems to function in the skin as a quencher of daylight, guarding human skin surface from fat peroxidation owing to contact to UV and other sources of ionizing emission.

Also, 9, 12 Octadecadienoic acids identified in n-hexane extract of *S. mombin* leaves in this study had been determined in methanol extract of *Nymphaea lotus* and *S. mombin* to have antimicrobial activity (Odumosu *et al.*, 2018). Qureshi *et al.* (2020) reported 9, 12 Octadecadienoic acids in n-hexane extract of *Kochia indica* stem. Likewise, Elufioye and Berida (2018) also reported 9, 12 Octadecadienoic acids in n-hexane extract of stem-bark and whole fruit of *S. purpurea.*

Alpha-amyrin identified in ethanol extract of *S. mombin* leaves in this study have been investigated in stem-bark of *Ficus exasperate* (Nogueira *et al.*, 2019). Guedes *et al.* (2020) also reported alpha-amyrin in hexane extract of *S. mombin* and *S. tuberose* leaves. In numerous studies, alpha-amyrin has established an abundant pharmacological applicability in some diseases (Melo *et al.*, 2010) that have a common feature in their development: inflammation as one of the key mechanisms involved in their origin (Ciccarelli *et al.*, 2013).

Gamma-sitosterol present in ethanol extract of this study had also been reported in roots of *Girardinia heterophylla* (Tripathiathi *et al.*, 2013). Qureshi *et al.* (2020) also reported the presence of gamma-sitosterol in n-hexane extract of *Kochia indica* stem. Gamma-sitosterol is a vital plant sterol and had been stated first time in *Girardinia heterophylla* (Tripathiathi *et al.*, 2013). Gamma-sitosterol decreases the hyperglycemia in Steptozotocin-induced diabetic rats owing to upsurge insulin production and inhibition of glucogenesis (Tripathiathi *et al.*, 2013). Sundarraj *et al.* (2012) also stated that gamma-sitosterol might affect the extent and activity of constituents of the extrinsic apoptotic pathway in human lung and breast adenocarcinoma cells. Ovuakporie-Uvo *et al.* (2018) also reported the presence of gamma-sitosterolin methanol extract of *Desplatsia dewevrei* fruit.

The beta-sitosterol identified in n-haxane extract of *S. mombin* leaves was also present in methanol extract of *Desplatsia dewevrei* leaves and fruit (Ovuakporie-Uvo *et al.*, 2018). Qureshi *et al.* (2020) also reported beta-sitosterol in n-hexane extract of *Kochia indica* stem. Its biological activities had been reported by authors. Villasenor *et al.* (2002) reported beta-sitosterol as an anthelminthic component of *Mentha cordifolia*. They employed *in vitro* tests by *Ascaris suum*, which resulted in the similar performance of worms treated with beta-sitosterol together with the positive controls, antiox and combantrin. Rahuman *et al.* (2008) also reported the reasonable larvicidal activity of five herbal drugs including: *Abutilon indicum, Solanum torvum, Jatropha gossypifolia, Euphorbia thymifolia* and *Aegle marmelos* on the larvae of *Culex quinquefasciatus*. Remarkably, the key isolated compound from the petroleum ether extract of *Abutilon indicum* was recognized as beta-sitosterol, which introduces this natural compound as a unique mosquito larvicidal sterol.

5. Conclusion

In conclusion, the study had been able to determine the possible chemical constituents of the ethanol and n-hexane extracts of *S. mombin* leaves using GC-MS technique. A number of compounds identified were found in abundance in the extracts. However, the percentage proportion of these compounds differed in each extract. In view of this, the possible biological properties of these identified compounds suggested that *S. mombin* might possibly be potential therapeutic agents.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declared no conflict of interest.

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