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Mini Review on Botany, Traditional Uses, Phytochemistry and Biological Activities of *Piper amalago* (Piperaceae)

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Piper amalago L. (Piperaceae) is widely distributed in Central and South America. It is commonly used in folk medicine as a diuretic and for the treatment of urinary calculus disease. This review highlights the findings of previously published studies on the botany, traditional uses, phytochemistry, and biological activities of *P. amalago*. The scientific information used for this review were obtained from various electronic databases including Science Direct, PubMed, Google Scholar, Scopus, and Web of Science. Amides represent the major chemical compounds that have been characterised in *P. amalago*. The essential oils, extracts, and isolated compounds of *P. amalago* were shown to possess antioxidant, insecticidal, antibacterial, anti-inflammatory, antileishmanial, cytotoxicity, acaricidal, anxiolytic, antilithiatic, antihyperalgesic, antinociceptive, antiarthritic, diuretic, wound healing, schistosomicidal, brine shrimp, cysteine protease, as well as neurobehavioral and toxicological activities. The outcomes of this review provide further support for the therapeutic potential of the genus *Piper*. Also, further research on the structure-activity relationship of some of the isolated phytochemicals may improve their biological potency and scientific exploitation of traditional uses of the *Piper* taxa.

Key words: Piperaceae; *Piper amalago*; phytochemistry; essential oil; amide

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The tropical plants of the Piperaceae family have provided many past and present civilizations with a source of diverse medicines and food-grade spices. This plant family has approximately 4000 species and is divided into five genera, namely *Piper*, *Peperomia*, *Lepianthes*, *Macropiper*, and *Trianopiper*. The genus *Piper* and *Peperomia* are the two most important genera of this family [1,2]. The genus *Piper* encompasses over 700 species that are widely distributed throughout the tropical and subtropical regions of the world [3]. Members of the *Piper* genus have commercial, economical, and medicinal importance. The genus, Piperaceae is also economically important as it is employed worldwide in the production of pepper in spice markets [4,5]. In addition, it is also used as remedies in many traditional medicinal systems such as the traditional Chinese and Indian Ayurvedic systems as well as folklore medicines of Latin America and West Indies.

Phytochemical studies have shown that plants of the genus *Piper* have several compounds such as amides, alkaloids, flavonoids, lignans, phenolics, terpenes, and essential oils [6-8]. *Piper amalago* L. is a herbaceous plant that grows in tropical and sub-tropical regions and mainly distributed in Mexico and Brazil. It is one of the most abundantly used plant due to its medicinal properties. This review aims to provide detailed information on *P. amalago*, particularly in terms of its botanical description, traditional uses, phyto-chemistry, and biological activities. An extensive body of literature comprising scientific journals derived from a variety of electronic databases, namely Science Direct, PubMed, Google Scholar, Scopus, and Web of Science has been employed in this review. It is envisaged that this review will provide a better understanding of *P. amalago* by highlighting its pharmacological potential in order to lay a foundation and provide reference for the follow-up research and wide application of the genus.

Botany

The Plant List contains 53 synonym plant names of *P. amalago* and recognises two varieties that contain the infraspecific taxa, namely *Piper amalago* var. *nigrinodum* (C.DC.) Yunck. and *Piper amalago* var. *variifolia* (Griseb.) Fawc. & Rendle [9]. *P. amalago*

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is native to South America including Argentina, Brazil, Bolivia, and Peru, the North of the Caribbean, Central America, and Mexico [10]. In Brazil, it is popularly known as jaborandi-manso, jaborandi-falso, and jaborandinhandi. In Mexico, it is known as kw'alaalits by the Huasteco natives [10]. It is described as an evergreen shrub or small tree that commonly grows to 1.5-3 meters in height, sometimes up to 6 meters. It is often grown in a moist or wet thicket, or mixed forest, at elevations of up to 2,600 meters. The stems are glabrous or minutely pubescent on the upper internodes. The petiole is generally slender (0.5-1 cm long) and can vaginate up to the middle, glabrous or pubescent; blade lanceolate to rounded or subobovate, 8-11 cm × 2.5-6.0 cm, apex acuminate, base equally attached to petiole, acute to subcordate, and glabrous or pubescent on veins. The inflorescences are erect, peduncle 0.8-1.5 cm long, glabrous; spike 6-7 cm long, not apiculate; rachis minutely pubescent; floral bracts cucullate, glabrous; thecae divergent, and dehiscing partially upward. The fruits were ovoid, conical to apex, 1.5-2 mm long, glabrous, or papillose [10,11].

Traditional Uses

P. amalago is used in traditional medicine from across Mexico to Brazil. The Yucatec Maya consumes a decoction for the treatment of snakebites [12]. In Brazil, it is traditionally used as a vermifuge [13] and diuretic for the treatment of urinary conditions [14]. Besides, the plant is frequently used in folk medicine for diuretic. It is also used the treatment of cardio-vascular problems such as coronary artery disease, congestive heart failure, atrial fibrillation, cerebrovascular disease, peripheral arterial disease, aortic aneurysm, and chronic kidney disease, as well as renal disorders such as chronic kidney disease, acute kidney injury, stones, infections, cysts, and cancer [15]. It is also used to alleviate chest pain and inflammation [16] and the tea from the leaves of *P. amalago* is used for the treatment of burns [17]. It has also been used to treat susto and epilepsy by the Q'eqchi Maya and other healers in Belize [18-20]. The leaves are crushed in water which is drank as a sedative. The liquid is also used to bathe the patient to treat the culture-bound syndrome *mal viento* or fatigue and sleeplessness. Nicaraguan midwives use a decoction of leaves and roots to reduce nervousness [21]. In Jamaica, the stems and leaves are boiled and consumed as a blood tonic [22].

PHYTOCHEMISTRY

The phytochemical properties of *P. amalago* have been explored since 1984. A total of 13 compounds have been isolated from the leaves and roots of *P. amalago*. The chemical structures are shown in Figure 1. The species is reported to comprise mainly of amides, which comprises of six pyrrolidines, three isobutyl amides, and one piperidine.

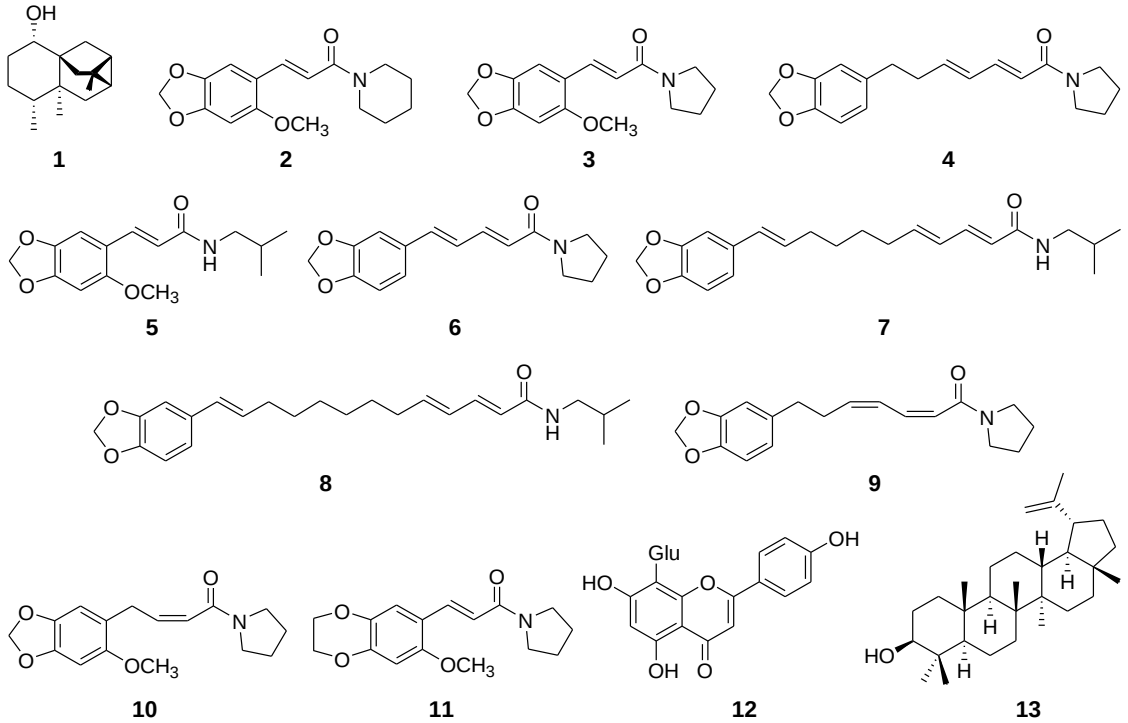

Figure 1. Chemical structures of isolated compounds from *P. amalago*

The first phytochemical study on *P. amalago* was performed by Achenbach et al. [23], who successfully isolated a sesquiterpene, ishwarol (**1**) from the root. A year later, Dominguez et al. [16] characterised the compounds, 2-methoxy-4,5-methylenedioxy-*trans*-cinnamoyl piperidide (**2**) and pyrrolidide (**3**). Meanwhile, Jacobs et al. [24] isolated six amides from the roots of Jamaican *P. amalago* which included *N*-[7-(3',4'-methylenedioxyphenyl)-2(*E*),4(*E*)-heptadienoyl] pyrrolidine (nigrinodine) (**4**), 2'-methoxy-4,5-methylenedioxy-*trans*-cinnamoylisobutyl-amide (**5**), trichostachine (**6**), pipericide (**7**), and guineensine (**8**), together with compound (**2**). It is worth noting that both compounds (**4**) and (**5**) were reported for the first time from this species. In another study, *N*-[7-(3',4'-methylenedioxyphenyl)-2(*Z*),4(*Z*)-heptadienoyl]-pyrrolidine (**9**) was successfully isolated using supercritical fluid extraction of methanol extracts from the leaves of *P. amalago* [25,26]. Additionally operational parameters involved in the extraction process of amide (**9**) using supercritical carbon dioxide were discovered [27]. Besides, Coelho et al. [28] successfully demonstrated the isolation of *N*-[3-(6'-methoxy-3',4'-(methylenedioxyphenyl)-2(*Z*)-propenoyl]pyrrolidine (**10**) and *N*-[3-(6'-methoxy-3',4'-ethylenedioxyphenyl) -2(*E*)-propenoyl]pyrrolidine (**11**). Rovani et al. [29] reported the presence of vitexin (**12**) and lupeol (**13**) from the leaves of *P. amalago.*

The chemical composition of *P. amalago* essential oils has been reported from various origins including Costa Rica [30], Ecuador [31], Guatemala [32], and Brazil [33-39]. Table 1 presents the chemical components identified from *P. amalago* essential oils collected from various origins. Monoterpenes hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes were reported as the major components of *P. amalago* essential oils. Germacrene D was characterised as the main component in the leaf and flower oils, constituting approximately 28.9% and 18.5%, respectively [30,33].

Additionally, bicyclogermacrene and α-amorphene were reported as the most dominant components of the *P. amalago* essential oils, collected from Brazil [33,34,36]. On the other hand, the leaf essential oils from Guatemala was found to be rich in (*E*)-nerolidol [32]. Similar results were also identified from the oils of the fruits collected in Brazil [37]. Furthermore, β-phellandrene, limonene, and β-copaen-4-α-ol were the only monoterpenoids present in high amounts from *P. amalago* essential oils [31,35,38,39].

Table 1: Chemical components identified from *P. amalago* essential oils

Country	Parts	Total identified		Major components (%)
		No	%	
Costa Rica	Leaves	39	100	Germacrene D (28.9%), β-elemene (24.6%), (E)-caryophyllene (20.8%), germacrene A (9.7%), bicyclogermacrene (4.4%) [30]
Ecuador	Leaves	38	90.16	β-Phellandrene (20.42%), spathulenol (10.34%), bicyclogermacrene (8.50%), α-pinene (7.29%) [31]
Guatemala	Leaves	13	99.5	(E)-nerolidol (48.7%), caryophyllene oxide (23.4%), spathulenol (7.5%) [32]
Brazil	Leaves	52	96.5	α-amorphene (25.7%), p-cymene (9.4%), (E)-methyl geranate (7.8%) [33]
	Flowers	28	95.5	germacrene D (18.5%), silphiperfol-6-ene (13.5%), limonene (10.5%) [33]
	Roots	48	99.0	α-amorphene (14.4%), α-muurolol (6.3%), α-gurjunene (4.4%) [33]
	Stems	51	94.1	α-amorphene (23.3%), α-muurolol (9.3%), longifolene (6.6%) [33]
Brazil	Leaves	136	99.54	Bicyclogermacrene (12.9%), limonene (6.1%), δ-cadinene (3.9%), α-pinene (3.7%) [34]
	Stems	89	98.60	bicyclogermacrene (12.0%), α-cadinol (9.4%), (Z)-caryophyllene (8.3%), τ-muurolol (8.3%), δ-cadinene (7.0%), (E)-nerolidol (5.2%) [34]
Brazil	Aerial parts	48	90.58	Limonene (20.52%), zingiberene (11.18%), δ-elemene (6.82%), α-pinene (5.23%), β-caryophyllene (4.69%) [35]
Brazil	Leaves	19	99.09	Biclyclogermacrene (27.91%), spathulenol (19.22%), germacrene D (9.94%), γ-muurolene (7.27%), α-cadinol (7.60%) [36]
Brazil	Ripe fruits	55	98.7	(E)-Nerolidol (19.9%), germacrene d-4-ol (12.7%), α-cadinol (8.2%), β-phellandrene (7.3%) [37]

	Unripe fruits	44	99.6	(E)-Nerolidol (14.2%), α-cadinol (11.1%), germacrene d-4-ol (10.3%), bicyclogermacrene (9.1%), β-phellandrene (8.2%), δ-cadinene (6.6%), epi-α-cadinol (6.1%) [37]
Brazil*	Leaves	28	NR	β-phellandrene (13.64%), (E)-nerolidol (8.08%), α-muurulene (7.85%), δ-elemene (6.42%), germacrene D-4-ol (5.54%), β-cedrene (5.15%) [38]
Brazil*	Leaves	NR	NR	β-copaen-4-α-ol (26%), 7-epi-α-eudesmol (21.84%), epi-α-cadinol (12.70%), n-hexyl-benzoate (12.29%) [39]

*no fulltext available; NR – not reported

BIOLOGICAL ACTIVITIES

Many pharmacological studies have been performed owing to the numerous traditional medicinal applications of *P. amalago*. The essential oils, extracts, and isolated compounds of *P. amalago* were shown to possess anti-oxidant, insecticidal, antibacterial, anti-inflammatory, antileishmanial, cytotoxicity, acaricidal, anxiolytic, anti-lithiasic, antihyperalgesic, antinociceptive, antiarthritic, diuretic, wound healing, schistosomicidal, brine shrimp, cysteine protease, neurobehavioral, and toxicological activities.

Antioxidant Activity

Caceres et al. [40] measured the antioxidant activity of *P. amalago* using total phenolics, DPPH, and ABTS assays. The methanol extract of *P. amalago* displayed IC_{50} values of 0.675 mg/mL (leaves) and 0.368 mg/mL (roots) using the DPPH assay, while the dichloro-methane extract gave IC_{50} values of 0.327 mg/mL (leaves) and 0.371 mg/mL (roots). For ABTS, the methanol extract demonstrated IC_{50} values of 0.370 mg/mL (leaves) and 0.509 mg/mL (roots), while the dichloromethane extract gave IC_{50} values of 0.392 mg/mL (leaves) and 0.351 mg/mL (roots). Meanwhile, the methanolic extract of the leaves revealed the highest phenolic content of 28.67 μg of gallic acid/mg extract.

Insecticidal Activity

The ethanolic leaf extract of *P. amalago* demonstrated a strong activity against adult *Drosophila melanogaster* Canton-S with an LC_{50} value of 27.95 mg/mL [41]. In another study, the ethanolic leaf extract showed potential for the control of against the tomato leafminer *Tuta absoluta* as it exhibited acute toxicity towards these caterpillars at a concentration of 2,000 mg/L, thus affecting the insect's development by reducing its survival and lengthening the larval and pupal stages. In constrast, the extract caused a significant lengthening of the larval and pupal stages at a concentration of 1,011 mg/L [42].

Wound Healing Effect

In a recent study, the aqueous extract of *P. amalago* leaves exhibited potential for wound healing a patient with type 2 diabetes mellitus. The topical application of the aqueous extract produced from the leaves of *P. amalago* facilitated the healing of a lacerated wound in the left thumb of the over a period of 15 days [43].

Antibacterial Activity

The methanolic extract of *P. amalago* revealed weak activity against *Escherichia coli* and *Staphylococcus aureus* with MIC values of 512 and 1024 μg/mL, respectively [44]. In addition, *P. amalago* essential oils displayed a moderate effect against *Staphylococcus aureus* and *Enterococcus faecalis* (MIC 100-200 μg/mL) and a weak antifungal effect against *Candida albicans* [31]. In another study, the leaf oil of *P. amalago* displayed weak antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli* with MIC values of 313, 1250, and 625 μg/mL, respectively [30].

Schistosomicidal Effects

The dichloromethane fraction of *P. amalago* leaf extracts showed significant activity against adult worms of *Schistosoma mansoni* at a concentration 100 μg/mL, thereby resulting in worm mortality (75%), separation of worm pairs (100%), and reduced motor activity (50%). On the other hand, no significant results were observed for other fractions of the stem extracts. In contrast, *N*-[7-(3',4'-methylenedioxy phenyl)-2(*Z*),4(*Z*)-heptadienoyl]-pyrrolidine (**9**) at a concentration 100 μM caused 100% mortality of adult worms after 24 h of incubation [13].

Anxiolytic Activity

The leaf extracts demonstrated significant anxiolytic activity in all the behavioral tests, with the strongest activity observed in the social interaction (SI) and conditioned emotional response (CER) paradigms. Besides, the ethanolic extract of *P. amalago* at a concentration of 66.5 μg/mL displaced 50% of the $GABA_A$–BZD receptor ligand [^{3}H]-Flunitrazepam [45] in an *in vitro* $GABA_A$ competitive binding assay.

Diuretic Activity

The oral administration of the ethanolic extract of *P. amalago* (125, 250, and 500 mg/kg) was found to significantly increase urine output after 24 h. The extract dose of 125 mg/kg was reported as the most

potent, with a diuretic index of 1.54. Equally important, the diuretic indexes were 1.34 and 1.32, respectively, for doses of 250 and 500 mg/kg [14].

Antilithiatic Activity

The ethanolic extract of *P. amalago* induced an inhibitory effect on calcium oxalate crystallisation in a turbidimetric model. The extract increased crystal density and produced smaller crystals based on the increased absorbance. For extract concentrations of 0.25, 0.5, and 1 mg/ml, an increase in the number of crystals was observed, consistent with the increased turbidity index values [14]. In another study, the essential oils of *P. amalago* leaves and stems showed highly significant results as compared to the control. However, there was a difference in the behaviour against the antilithiatic activity between these two oils. The leaf oil was found to be more active, with its maximum activity level observed at 5 mg/mL and a constant behaviour displayed across the different concentrations tested. In contrast, the stem oil extracts displayed different activity levels at various concentrations and obtained maximum activity at 2.5 mg/mL [34].

Neurobehavioral and Toxicological Activities

The LD_{50} of *P. amalago* extract in rats was estimated to be 2,545 mg/kg i.p. with an observation period of 14 days. Nevertheless, in the open field behaviour of rats, no significant differences were observed among the control groups and groups that received *P. amalago* in all of the parameters observed. In the elevated plus-maze test, the extracts (250 or 420 mg/kg) significantly increased the time spent in closed arms as compared to saline, thus suggesting an anxiogenic effect of these species. However, in the comet assay, *P. amalago* extract did not show any genotoxic effect on the blood, liver, and brain tissues of the rats. In the micronucleus test, there was no significant difference in the frequency of micronuclei in any of the groups tested, thereby suggesting that the extracts did not induce mutagenic activity [46].

Other Biological Activities

The leaf oil of *P. amalago* showed notable brine shrimp lethality assay against *Artemia salina* with an LC_{50} value of 4.64 µg/mL [30]. The leaf oil of *P. amalago* also showed cruzain inhibitory activity against *Trypanosoma cruzi* cells with an IC_{50} value of 68.9 µg/mL [30]. The *P. amalago* essential oil was found to be inactive against the newly hatched larvae of the cattle tick, *Rhipicephalus (Boophilus) microplus*, with an LC_{50} value >10 µg/mL [35]. The ethanolic extract of *P. amalago* inhibited mechanical hyperalgesia, knee edema, and heat hyperalgesia, but not depressive-like behaviour induced by the intraplantar injection of the Complete Freund's adjuvant (CFA) model. When evaluated using the spared nerve injury (SNI) model, the extract inhibited mechanical and cold hyperalgesia. In addition, the extract and *N*-[7-(3',4'-methylenedioxyphenyl)-2(*Z*),4(*Z*)-heptadienoyl]-pyrrolidine (**9**) and *N*-[7-(3',4'-methylenedioxyphenyl)-2(*E*),4(*E*)-heptadienoyl]pyrrolidine (nigrinodine) (**4**) prevented the mechanical hyperalgesia induced by carrageenan and the anti-nociceptive effects in both phases of formalin nociception. However, the extract did not induce any alterations in the open field test [47].

The chloroform leaf extract of *P. amalago* demonstrated topical anti-inflammatory activity against the Croton oil-induced ear edema in mice with an ID_{50} value of 498 mg/cm^2. In contrast, the hexane and methanol extracts showed weak activity with ID_{50} values >1000 mg/cm^2 [48]. The leaf extract of *P. amalago* showed strong activity against the promastigotes and intracellular amastigotes forms of *Leishmania amazonensis* with IC_{50} values of 16 and 7 µg/mL, respectively. In addition, *N*-[7-(3',4'-methylenedioxyphenyl)-2(*E*),4(*E*)-heptadienoyl]pyrrolidine (nigrinodine) (**4**) exhibited strong activity against the promastigote and intracellular amastigote forms with IC_{50} values of 15 µM and 14.5 µM, respectively [25]. The leaf extract of *P. amalago* showed cytotoxicity against the J774A1 macrophage cell line with a CC_{50} value of 93 µg/mL [25]. In another study, the leaf oil of *P. amalago* showed cytotoxic activity against MCF-7 breast tumour cells with an IC_{50} value of 52.9 µg/mL [30].

CONCLUSION

In this review, the knowledge on botany, traditional uses, phytochemistry and biological activities of *P. amalago*, which has been widely used to treat several illnesses, has been summarised. *P. amalago* has been demonstrated to be reliable and it is therefore important to determine if modern pharmacological studies on *P. amalago* are available to assess its traditional uses. Some of the modern *in vitro* and *in vivo* biological studies highlighted in this review have confirmed the traditional uses of *P. amalago*. Based on the current information, amides have been isolated mainly from *P. amalago* extracts. However, there are existing gaps in the scientific studies performed on *P. amalago*. Firstly, phytochemistry studies on the structural characterisation of metabolites in *P. amalago* are very limited. Thus, in-depth studies on the structural identification and purification of bioactive metabolites from *P. amalago* is essential. Secondly, insufficient pharmacological studies have been conducted on *P. amalago*. For instance, some biological activity studies lacked comparison with standard positive and negative controls, thereby possibly leading to false positive results.

Moreover, previous pharmacological investigations were mostly focused on the organic fractions of the crude extracts with little attention on the aqueous extracts which have been traditional used. Likewise, clinical trials are also required to evaluate its efficacy and safety. Thirdly, although *P. amalago*

possesses various potential therapeutic effects, these studies were only performed using animal and cell models and clinical investigations using humans have rarely been implemented. Hence, future investigation should be focused on the bioactivity of *P. amalago* in various clinical studies involving humans for the assessment of beneficial pharmaceutical applications of *P. amalago*.

Overall, the presence of biologically active molecules such as amides and sesquiterpenoids identified as major components in *P. amalago* serves as a potential natural source of compounds which can be used in the food, aromatics, cosmetics and pharmaceutical sectors. It is envisaged that more studies on the bioavailability and pharmacokinetics of *P. amalago* are required to identify the metabolites responsible for its bio-activities. To date, the available pharmacological studies are insufficient to assess the ethnobotanical uses of *P. amalago*. It is hoped that the information presented in this review will increase the awareness of *P. amalago* and provide more avenues for further research on their medical applications.

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