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From Instinct to Innovation – Smart Herb Hunting: A Mini-review on Ethological cues, Ethnomedicine, and Emerging Modalities

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Abstract

Zoopharmacognosy – a subspeciality of ethology – deals with the study of non-human animal selection and use of plant parts, fungi, and natural products to prevent and cure their diseases (Alves and Rosa, 2013). The mini-review positioned at the intersection of evolutionary ecology, ethology, and ethnomedicine is a preliminary attempt to synthesise evidence in favour of the identification of potential traditional medications by deriving cues from animal-plant interactions across the animal taxa. Self-medication is employed by different animals instinctively as well as through learned behaviour. It is observed in diverse forms of organisms from *Formica paralugubris* (wood ants) for use of nest building material derived from coniferous resins for its antimicrobial properties to the first report of ingestion of bitter pith sap of *Vernonia amygdalina* to get rid of the parasitic nematodes by great apes (Chimpanzees- *Pan toglodytes*, Bonobos- *Pan panicus*, and Gorilla- *Gorilla gorilla*) in Sub-Saharan Africa (Castella et al., 2008; Huffman M.A., 2003). These evidences support both therapeutic and prophylactic approaches derived from olfactory and sensory signals. However, the development of potential drugs requires rigorous authentication, including performing toxicity studies, phytochemistry (using HPLC, GC-MS, LC-MS/MS, NMR, etc.), cyto-genotoxicological studies, and computational approaches such as ADMET candidate drug prediction. New modalities include the use of artificial intelligence and machine learning in ethography, untargeted meta-blome analysis, and computational methods for identifying potential drug candidates. The synthesis is an attempt to support animal self-medication as an evolutionarily grounded, practically viable framework for identifying safe candidate drugs from ethnomedicine.

Keywords: Non-human self-medication, Zoopharmacognosy, Traditional medicine safety, Phytochemical validation.

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Introduction

The term zoopharmacognosy was popularized and consolidated by Huffman (1997, 2003) to describe the instinctive and learned behaviour of non-human animals to self-medicate using plants, soil, fungi, and natural products in a prophylactic and therapeutic manner. The concept is grounded in evolutionary ecology, ethological cues, pharmacology, and ethnobotany in the search for potential drug biomolecules through the animal-plant interactions (Mezcua Martin et al., 2019; Alves & Rosa, 2013). In a nutshell, it encapsulates the millions of years of co-evolutionary animal-plant interaction. It represents the refinement of potential biomolecules from the secondary metabolite pool through whole-organism-bioassay – a system which cannot be replicated at such a scale in any laboratory.

The early naturalist observed and noted that animals sought specific plant-based remedies to cure themselves. However, comprehensive historical documentation of self-medication began in the early 20th century with the beginning of integrative interdisciplinary studies in ethology, ethnobotany, and primatology (Huffman, 1997; Lozano, 1998). The classic case of Tanzanian female chimpanzee, Chausiku, chewing the bitter pith of the non-nutritional plant *Vernonia amygdalina* at Mahale Mountain National Park, has initiated a series of integrative interdisciplinary research in this field, which continues to date (Huffman and Shifu, 1989). Since then, zoopharmacognostic cues have been documented across different animal taxa, including insects, birds, and great apes, and have fundamentally changed our assumptions that self-care is limited to humans.

This mini-review synthesises self-medicative events across the animal kingdom and is a preliminary attempt to pool evidence from ethological, ethnomedicinal, and pharmacological dimensions. It also systematically enumerates phytochemical validation, computational methods such as ADMET candidate molecule predictions, cytogenotoxicological studies, and new modalities, including ethography using artificial intelligence and machine learning for putative drug leads.

Self-medication Across the Animal Kingdom

Self-medication is reported in nearly all the major animal phyla – from Hymenopterans to Hominids, depicting that it is evolutionarily widespread and not exclusive to primates (De Roode, 2013). Lozano (1998) recognised two functional types: prophylactic and therapeutic

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self-medication; the former is a preventive measure adopted even before the appearance of symptoms, and the latter is taken by a visibly sick or inactive member of a group.

Huffman (2001), based on his field observations, postulated six self-medication-related criteria to be satisfied for classifying an event as self-medication, as follows:

(1) Non-nutritional consumption, the plant is not the part of normal nutritional diet.
(2) The intervention is adopted by a symptomatic member of a group with visible symptoms or inactivity and is verifiable with documented observations and correlation with health status.
(3) The intervention is adopted for a specific episode of infection irrespective of the year-long availability of the plant.
(4) The ingestion has subsequent clear signs of physiological recovery.
(5) The selected plant has verifiable documented bioactive principles known for the condition or ailments, intervention for which is adopted by the animal.
(6) Unusual processing of the selected plant part for example chimpanzees in Mahale mountain, Tanzania, carefully tear apart the upper bark of *Vernonia amygdalina* and chewed the pithy central part sucking the bitter pith and spitting out the fibrous part, which is not used for normal foraging.

Similarly, to classify a behavior as self-medicative, De Roode et al. (2013) proposed the following five criteria that must be met to qualify it as a therapeutic self-medication:

(1) Ingestion or topical application of the non-nutritional plant or natural product.
(2) The episode of ingestion or initiation caused by an active infection.
(3) The ingestion or application reduces the pathogen load and improves its health parameters and overall fitness.
(4) It is an expensive behavior in terms of toxicity, if not so, every individual would have taken it without exception.
(5) It is observable in the natural habitat of animal, not only in captive conditions.

Representative examples from various groups of animal taxa are documented in Table 1.

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Table 1: Examples of Self-medicative Events Across Various Taxa of Animals

S. No.	Group	Zoological Name	Plant Material Used	Active Principle(s)	Function	References
1.	Bird (Passeriformes)	*Sturnus vulgaris* (European starling)	*Daucus carota* (Wild carrot), *Erigeron philadelphicus*	Volatile compounds- terpenes, phenolics, sesquiterpenes,	Nest fumigants to lower the ectoparasitic mite burden	Clark & Mason (1988)
2.	Mammal (Primates)	*Pan troglodytes* (Chimpanzee)	*Vernonia amygdalina* bitter pith consumption	Vernodalin, vernolide, hydroxyvernolide	Therapeutic nematocidal	Huffman & Seifu (1989); Koshimizu et al. (1994)
3.	Insect (Hymenoptera)	*Formica paralugubris* (Wood ants)	Coniferous resin	Sesquiterpenes, monoterpenes	Anti-microbial nest protectant (Prophylactic)	Castella et al. (2008)
4.	Insect (Lepidoptera)	*Dinaus Plexippus* (Monarch buttrefly)	*Asclepias curassavica* (Tropical milkweed)	Higher cardenolide content than the *A. incarnata*	Transgenerational medication, infected females preferentially lay eggs on high cardenolide plant species to reduce the spore load	Lefèvre et al. (2010)
5.	Insect (Diptera)	*Drosophila melanogaster* (Fruit fly)	Rotting fruits rich in alcohol (Due to fermentation)	Ethanol	Infected larvae seek ethanol for killing the parasitoid wasp larvae in the haemocoel	Milan et al. (2012)
6.	Insect (Hymenoptera)	*Apis mellifera* (Honey bee)	Propolis accumulation (Plant resin) flavonoids, terpinoids)	Flavonides, terpinoids, polyphenols	Antifungal attributes	Simone-Finstrom & Spivak (2012)
7.	Mammal (Artiodactyla)	*Ovis aries*/ *Capra hircus* (Sheep/ Goat)	Sanfoin, tannin-rich foliage	High concentration of tannins	Selective foraging on tannin rich forage under helminth parasite load	Villalba et al. (2014)

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S. No.	Group	Zoological Name	Plant Material Used	Active Principle(s)	Function	References
8.	Mammal (Proboscidea)	*Elephas maximus* (Asian elephant)	Multiple plant species for different conditions according to Karen mahout eg. Roots of *Lithocarpus polystachyus* in Diarrhea	Not documented in the reference paper	Self-medication events documented form experience of Karen mahouts in Thailand which they use in ethnoveterinary practices	Green et al. (2020)
9.	Mammal (Primates)	*Leontopithecus chrysopygus* (Black lion tamarin) + nine mammal species	cabreúva balsam (*Myroxylon peruiferum*), fur rubbing on trunk and application of resin	Anti-ectoparasitic resinous balasam (No active principle documented in the paper)	Black tamarin rub resin/ balsam on abdominal, thoracic, and inguinal region to repel ectoparasites	Kaisin et al. (2022)
10.	Mammal (Primate)	*Pongo abelii* (Sumatran orangutan)	*Fibraurea tinctoria*, chewed leaves topical application	Furanoditerpinoids, prtoberberine alkaloidso	Analgesic, antipyretic, diuretic, and wound healing properties	Laumer et al. (2024)

Behaviour-driven Selection of Plant Species

Animals use their olfactory and sensory cues, refined over long evolutionary mechanisms, to identify plants with bioactive principles for self-medication against the daily dietary selection. Olfactory signals involve a unique profiling mechanism that characterises odours and their associated relief, achieved through repetition of the event if needed, or through innate olfactory mechanisms (Villalba & Provenza, 2007; De Roode et al., 2013).

Animals use regulatory feedback mechanisms to distinguish a therapeutic and harmful toxic level of bioactive bitter principle for titration of the requisite dosage. Villalba and Provenza (2007) demonstrated that the cattle start foraging on a tannin-rich diet under a severe load of enteric parasites and gradually taper and shift to their normal foraging pattern as the infection

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subsides or is ultimately resolved by a homeostatic feedback mechanism. This homeostatic feedback mechanism is also known in great apes and is thought to be a precursor of the pharmacological dosing mechanism in humans (Villalba et al., 2014).

Huffman and Vitazkova (2007) proposed four levels of zoopharmacognostic behaviours:

(1) Avoidance of a source of infection or toxic foraging material.
(2) Selection of healthy dietary material rich in nutrition to remain healthy.
(3) Curative ingestion of specific plant species with active principles.
(4) Direct topical application of the chewed leaf paste.

The first two are classified as passive interventions, whereas the last two are considered active interventions.

Zoopharmacognosy vs Ethnomedicine

Grade et al. (2009) in their study on self-medication among pastoralist livestock in Karmoja, Uganda, found 72% concordance in the treatment of human and veterinary ailments. This empirical evidence suggests that it is the result of both humans and other animals interacting with the same plant communities for millennia, as also envisioned by Huffman (2003). This alignment in the selection of the same plant species by both humans and animals has zoopharmacognostic translational value for identifying potential pharmacologically significant biomolecules, rather than relying on random screening without these aids. Karen mahouts in Thailand directly use the plant selection of elephants in their ethnoveterinary knowledge to treat their livestock, supporting the view that ethological cues get translated into an ethnoveterinary knowledge base (Greene et al., 2020).

Similarly, the self-medication by Tanzanian chimpanzees for intestinal parasites using plant species- *Vernonia amygdalina*, also has a place in Sub-Saharan traditional medicine systems for the treatment of parasitic infections, intestinal ailments, and pyrexia (Huffman & Seifu, 1989). Later phytochemical characterisation of the plant by Koshimizu et al. (1994) yielded antiparasitic sesquiterpene lactones- vernodalin, vernolide, and hydroxyvernolide, supporting interdisciplinary studies in zoopharmacognosy, animal behaviour, and ethnomedicine.

Thus, zoopharmacognosy not only provides new leads to putative drug molecules but also a dual-filter mechanism: animal selection as evolutionary pre-screening and ethnomedicine may provide long-term human safety; the latter needs multi-tier validation to identify potential therapeutic molecules.

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Phytochemical Validation and new Modalities

Zoopharmacognostic cues are primary and the first step in the identification of a putative drug candidate. It follows a rigorous multi-tier pipeline of phytochemical profiling, cytogenotoxicity evaluation, and computational methods, etc., for the screening of active plant secondary metabolites with therapeutic potential (Calixto, 2000). The main validation approaches, along with new modalities, are briefly described in Table 2.

Table 2: Validation pipeline

Tier(s)	**Methodologies/ Techniques**	**Expected outcome**	**References**
Phytochemical analysis	HPLC, GC-MS, LC-MS/MS, NMR spectrophotometry etc.	Identification and quantitative assessment of the active principle used in self-medication.	Koshimizu et al., 1994; Calixto, 2000; Sarkar et al., (2024); Ekeanyanwu et al., (2024)
In-silico pre-screening	ADMET prediction, molecular docking, Lipinski Ro5 analysis, network pharmacology,	Before moving into *in-vivo* trials, prefiltration of putative molecules for their bioavailability, drug-likeliness, low-toxicity profiling, and target identification, etc.	Dominguez-Martin et al. (2020); Sarkar et al., (2024).
Acute toxicity studies	*In vivo* model organism-based single-dose LD_{50} study as per the OECD guidelines	For the initial safety assessment, the determination of LD_{50} using crude extract and pure compound.	Legba et al. (2019); Sarkar et al. (2024); Ariyo et al. (2024); Freymann et al. (2024).
Sub-chronic and chronic toxicity studies	Repeated *in vivo* dosage studies as per OECD guidelines for 28-90 days to 6-12 months	Histopathological profiling using NOAEL and LOAEL for meeting the regulatory norms	Adeneye et al. (2014); Akindele et al. (2014); Legba et al. (2019); Ekeanyanwu et al. (2024).
Cyto-genotoxicity studies	Ames test, MTT assay, comet assay, micronucleus test	Evaluation of cyto-genotoxicity risks	Adeneye et al. (2014); Akindele et al. (2014); Senes-Lopes et al. (2023); Ariyo et al. (2024).
Ethography using AI and ML	Application of AI, ML, and CNN for real-time analysis of self-medicative	High-throughput literature screening and fast automated zoopharmacognostic inputs	New modalities- It is part analogous to PhenoCam-based studies and use of digital herbarium data

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	events in the field and literature	for faster evidence synthesis	in plant phenological studies.
Untargeted meta-blome analysis	LC-MS/MS, GC-MS, 16S rRNA amplicon sequencing	Maps how plant secondary metabolites interact with the animal gut to uncover new drug molecules and their mode of action.	Gregor et al. (2022)

Abbreviations: HPLC= *High Performance Liquid Chromatography*; GC-MS= *Gas Chromatography- Mass Spectrometry,* LC-MS/MS= *Liquid Chromatography- Tandem Mass Spectrometry*; NMR= *Nuclear Magnetic Resonance spectroscopy*; ADMET= *Absorption, Distribution, Metabolism, Excretion, and Toxicity*; Ro5= *Lipinski's Rule of Five*; MTT= 3-(4,5-*Dimethylthiazol-*2-*yl*)-2,5-*Diphenyltetrazolium Bromide* (*Cell viability assay*); LD_{50}= *Median Lethal Dose*; OECD= *Organisation for Economic Co-operation and Development*; NOAEL= *No Observed Adverse Effect Level*; LOAEL= *Lowest Observed Adverse Effect Level*, 16S rRNA= *16S ribosomal Ribonucleic Acid* (*Marker gene*)

Conclusion

Zoopharmacognosy is an ancient, evolutionarily widespread concept that includes ecology, comparative ethology, ethnomedicine, and pharmacognosy, etc. In this minireview we examined transgenerational self-medication adopted prophylactically by monarch butterfly (*Danaus plexippus*) by preferentially laying eggs on tropical milkweed species of its choice (*Asclepias curassavica*) to reduce the spore load of *Ophryocystis elektroscirrha*, European starling (*Sturnus vulgaris*) building nest from plant derived fumigating materials (Wild *Daucus carota*, *Erigeron philadelphicus*), Sumatran orangutan treating its facial wound with natural ointment made from the leaf paste of *Fibraurea tinctiora,* etc., collectively supporting that the concept of self-medication is evolutionarily grounded, result of millions of years coevolution and animal-plant interaction.

Huffman (2003) argued that the pharmacopoeia derived from such zoopharmacognostic studies may hold huge potential for the identification of putative drug biomolecules. The challenges and opportunities include establishing such systematic wild-animal-derived-pharmacopoeias by using new aids such as the use of artificial intelligence and machine learning in ethography, modern computational approaches, cyto-genotoxicity studies, comprehensive safety validation, and dose translation.

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