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Plant Secondary Metabolites—A Necessary Resource for Both Man and Papilionid Butterflies Across West Bengal, India †

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Abstract: Chemical factors or plant secondary metabolites (PSM) contributes towards larval host plant choice among butterflies. Significantly, such PSM are the basis of ethnomedicinal plants as recognised by traditional healers since time immemorial. A total of 27 papilionid butterflies utilizing 26 species of medicinal plants as their ovipositing substrate were observed. *Graphium cloanthus, G, sarpedon, G, eurypylus* (PI values: 3.873) were polyphagous species. On the contrary, *G. antiphates, G. nomius, Atrophaneura polyeuctes, A. aidenous, Troides helena*, (PI: 1.000) were strictly monophagous. The medicinal utilization values (U_v) of such plant species was recognised. *Aristolochia indica* (U_v=0.474) and *Citrus medica* (U_v=0.44) appeared to be the most popular medicinal plant among the proponents of ethnomedicine.

Keywords: butterflies; ethnomedicine; plant secondary metabolites; polyphagy; medicinal utilization values

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Introduction:

Indigenous knowledge is known to generate an inexhaustible information database for its exponents. Such has been the contribution of medicinal plants towards the well being of its practitioners since centuries. The popularity associated with such plants have been mentioned even in Charaka Samhita, Susruta Samhita, Rig-Veda, Astanga Hridaya, Ramayana and Mahabharata [1,2]. WHO has reported the utilization of traditional medicines amongst 80% populace from developing nations [3]. Plant secondary metabolites (PSM) or more specifically alkaloids, terpenoids, flavonoids, glycosides, coumarins, saponins and tannins constitutes the basis of such formulations as used by folklore therapists. Their immense popularity thereby creates an urgent demand for the conservation and judicious utilization of these plant resources.

Interestingly, butterflies have developed an innate skill to recognise and utilize such plants as reliable ovipositing substrate. Several species have devised strategies to overcome the defensive substances (PSM) characteristics of plant taxa [4-6]. Although being totally dependant on floral resources as adult, the demand for tender foliage in their larval stage cannot be overlooked. Butterflies thus exhibit a "botanical instinct" in selecting the most perfect host plant suitable for egg deposition [7]. As larvae butterflies are known to exhibit host plant specificity by being monophagous, polyphagous or oligophagous. Significantly such chemical constituents (PSM) are critically important in establishing an intrinsic butterfly-host plant relationship.

Thus the present study would attempt to explain the significance of PSM in the life of butterflies as well as an essential element of ethnomedicine. The determination of medicinal utilization value (U_{vs}) for each studied plant species would also be helpful in deciphering their extent of therapeutic benefits. Additionally the degree of polyphagy would be investigated with an attempt to generate an idea of larval food plant specificity of butterflies.

Material and Methods:

Study Design

The entire study was conducted between June 2019 to May 2020 across tribal dominated districts of West Bengal (East and West Midnapore, Darjeeling, Jalpaiguri, Purulia and Bankura), India. The initial phase of study involved documentation of papilionid butterflies utilizing medicinal plants as their larval resource. Several instances of larvae feeding on plants belonging to one or more plant order or family or genera or species were observed for determining their monophagous / polyphagous nature.

Identification of butterfly larval host plants up to species was conducted from specially designed quadrats laid for such purpose. On field identification of plants was done based on earlier reports in most instances along with direct observation of larvae during such survey. Such observations were later confirmed from published literature [8-10]. Additionally plant species were also identified from previous publications [11-17].

In the final phase of investigation, detailed ethno-medicinal survey was undertaken to emphasise the therapeutic benefits of such plants. This involved an informal dialogue designed to gather information from knowledgeable and experienced populace including ojhas, kabiraaj and vaidyas. Such people were initially interviewed based on specially designed questions prepared for such purpose, followed by group discussion and informal meeting whenever required. Information thus obtained were documented in datasheet without any modification.

Data Analysis:

Polyphagy as evident among butterfly species was determined using polyphagy index (PI) where PI= $(A\times B)^{1/2}$; where A= number of genera of host plants, and B=taxonomic diversity of the hosts (1=one plant species; 2=one plant genus; 3=one plant family; 4= one plant order; 5= two or more plant orders) [18].

Estimation of medicinal values (U_{vs}) of each species "s" was done based on the following:[19-20]. The formulae used was $U_{vs}=\Sigma U_s/n_s$ where U_s in the number of medicinal uses of the species "s" as mentioned by informant and n_s was the number of informants who provided the information.

Results:

A total of 27 papilionid species utilizing 26 species of medicinal botanicals as their larval food resource was identified across districts of West Bengal, India. Five plant families i.e. Rutaceae (38.46%), Lauraceae (19.23%), Magnoliaceae and Annonaceae (15.38%) and Aristolochiaceae (11.54%) were reported as larval food resource of butterflies (Table 1).

Table 1. List of papilionid species along with their ovipositing plants and polyphagy index.

Sr. No.	Papilionid larval species	Ovipositing plant species	Polyphagy Index (PI)
1	*Graphium cloanthus* Westwood	**Family: Magnoliaceae** *Michelia champaca* Linn. *Michelia doltsopa* Buch.-Ham. ex DC. **Family: Lauraceae** *Cinnamomum camphora* (L.) J. Presl. *Persea odoratissima* (Nees) Kosterm.	3.873
2	*Graphium sarpedon* (Linnaeus)	**Family: Annonaceae** *Polyalthia longifolia* Hk. f. & T.	3.873

		Family: Lauraceae *Cinnamomum camphora* (L.) J. Presl *Persea odoratissima* (Nees) Kosterm	
3	*Graphium eurypylus* (Linnaeus)	**Family: Annonaceae** *Annona reticulata* L *Polyalthia longifolia* Hk. f. & T. **Family: Lauraceae** *Cinnamomum camphora* (L.) J. Presl	3.873
4	*Graphium chironides* (Honrath)	**Family: Magnoliaceae** *Magnolia pterocarpa* Roxb.	1.000
5	*Graphium doson* (C.& R. Felder)	**Family: Magnoliaceae** *Michelia champaca* Linn *Magnolia grandiflora* L **Family: Annonaceae** *Polyalthia longifolia* Hk. f. & T.	3.464
6	*Graphium agamemnon* (Linnaeus)	**Family: Magnoliaceae** *Michelia champaca* Linn **Family: Annonaceae** *Annona squamosa* Linn *Annona reticulata* L *Polyalthia longifolia* Hk. f. & T	3.464
7	*Graphium antiphates* (Cramer)	**Family: Annonaceae** *Uvaria lurida* Hk. f. & T.	1.000
8	*Graphium nomius* (Esper)	**Family: Annonaceae** *Polyalthia longifolia* Hk. f. & T	1.000
9	*Chilasa epycides* (Hewitson)	**Family: Lauraceae** *Cinnamomum camphora* (L.) J. Presl *Persea odoratissima* (Nees) Kosterm.	2.449
10	*Chilasa agestor* (Gray)	**Family: Lauraceae** *Cinnamomum tamala* Nees. *Persea odoratissima* (Nees) Kosterm.	2.449
11	*Chilasa clytia* (Linnaeus)	**Family: Lauraceae** *Litsæa glutinosa* (Lour.)C.B.Rob. *Cinnamomum camphora* (L.) J. Presl *Cinnamomum tamala* Nees.	2.449
12	*Chilasa slateri* (Hewitson)	**Family: Lauraceae** *Litsæa glutinosa (*Lour.)C.B.Rob. *Litsæa polyantha* Juss. *Cinnamomum camphora* (L.) J. Presl	2.449
13	*Papilio helenus* Linnaeus	**Family: Rutaceae** *Glycosmis pentaphylla* Correa. *Toddalia asiatica* Lamk.	2.449
14	*Papilio polytes* Linnaeus	**Family: Rutaceae** *Glycosmis pentaphylla* Correa. *Citrus grandis* (L.) Osbeck *Citrus limon* (L.) Burm. f. *Aegle marmelos* Correa. *Citrus medica* Linn. *Murraya koenigii* Spreng.	3.464
15	*Papilio castor* Westwood	**Family: Rutaceae** *Glycosmis pentaphylla* Correa. *Toddalia asiatica* Lamk.	2.449
16	*Papilio memnon* Linnaeus	**Family: Rutaceae** *Citrus grandis* (L.) Osbeck *Citrus limon* (L.) Burm. f. *Citrus medica* Linn.	1.414

17	*Papilio polymnestor* Cramer	**Family: Rutaceae** *Glycosmis pentaphylla* Correa. *Citrus grandis* (L.) Osbeck *Citrus aurantium* Linn.	2.449
18	*Papilio demoleus* Linnaeus	**Family: Rutaceae** *Glycosmis pentaphylla* Correa. *Citrus grandis* (L.) Osbeck *Aegle marmelos* Correa. *Citrus medica* Linn.	3.000
19	*Papilio krishna* Moore	**Family: Rutaceae** *Citrus medica* Linn. *Zanthoxylum acanthopodium* DC.	2.449
20	*Papilio paris* Linnaeus	**Family: Rutaceae** *Citrus medica* Linn. *Toddalia asiatica* Lamk.	2.449
21	*Papilio polyctor* Boisduval	**Family: Rutaceae** *Zanthoxylum acanthopodium* DC. *Clausena excavata* Burm.	2.449
22	*Atrophaneura polyeuctes* (Doubleday)	**Family: Aristolochiaceae** *Aristolochia tagala* Cham. & Schlect.	1.000
23	*Atrophaneura aristolochiae* (Fabricius)	**Family: Aristolochiaceae** *Aristolochia tagala* Cham. & Schlect. *Aristolochia indica* L.	1.414
24	*Atrophaneura hector* (Linnaeus)	**Family: Aristolochiaceae** *Aristolochia tagala* Cham. & Schlect. *Aristolochia saccata* Wall.	1.414
25	*Atrophaneura varuna* (White)	**Family: Aristolochiaceae** *Aristolochia tagala* Cham. & Schlect. *Aristolochia saccata* Wall.	1.414
26	*Atrophaneura aidoneus* (Doubleday)	**Family: Aristolochiaceae** *Aristolochia saccata* Wall.	1.000
27	*Troides helena* (Linnaeus)	**Family: Aristolochiaceae** *Aristolochia tagala* Cham. & Schlect.	1.000

G. cloanthus, G, sarpedon, G, eurypylus (PI values: 3.873) were widely popular polyphagous species. Interesting, observation on *G. doson, G. agamemnon* and *P. polytes* (PI= 3.464) were noted (Table 1). While *G. doson* and *G. agamemnon* were recognised for utilizing plants belonging to single plant order, *P. polytes* utilized multiple species belonging to a single family (Rutaceae). On the contrary, *G. antiphates, G. nomius, A. polyeuctes, A. aidenous, T. helena*, (PI: 1.000) were strictly monophagous (Table 1).Other notable observation on the medicinal utilization values (U_{vs}) of the papilionid larval plant species revealed some interesting findings (Table 2). *A. indica* (U_v=0.474) and *C. medica* (U_v=0.44) appeared to be the most popular among folklore therapists. However *M. champaca* (0.39), *C. camphora* (0.37), *G. pentaphylla* (0.37), *C. aurantinum* (0.380) *and A. tagala* (0.36) with $U_v \geq$ 0.35 were also popular among traditional healthcare practitioners (Table 2).

Table 2. List of medicinal plant species along with their pharmacological benefits and medicinal utilization values.

Sr. no.	Plant species	Pharmacological benefit	Utilization values (U_v)
1	**Family: Magnoliaceae** *Michelia champaca* Linn**.**	Anti-inflammatory, analgesic, nerve soothing effect, treatment of skin diorders, gout. Effective in aromatherapy	0.39

2	*Michelia doltsopa* Buch.-Ham. ex DC.	Treatment of dyspepsia, gonorrhea, stomach disorders, tonsillitis, fever. Also useful as deworming agent	0.31
3	*Magnolia pterocarpa* Roxb.	Effective in treatment of fever and cough	0.30
4	*Magnolia grandiflora* L	Antiinflammatory, diaphoretic, stimulant, prevention against cold, headache and stomach ache	0.32
5	**Family: Lauraceae** *Cinnamomum camphora* (L.) J. Presl	Camphor used to treat high fever, measles, delirium, whooping cough, melancholia, bronchitis, uterine pain, asthma, gonorrhoea, rheumatism, headache and influenza	0.37
6	*Cinnamomum tamala* Nees.	. Beneficial for the treatment of itch, diarrhoea, colic, rheumatism, fever, anaemia, nausea and vomiting	0.30
7	*Persea odoratissima* (Nees) Kosterm.	Anti-inflammatory, antiseptic, antiviral, anti-allergic, effective againest snakebite, burn wounds, improves cardiac output, reduces congestive heart failure and cardiac arrhythmia	0.30
8	**Family: Annonaceae** *Polyalthia longifolia* Hk. f. & T.	Treatment of fever, skin disease, diabetes, hypertension, constipation. Known for its anti-inflammatory ,anticancer, antimicrobial, antiulcer activity	0.32
9	*Annona reticulata* L	Treatment of epilepsy, dysentery, cardiac problem, worm infection, constipation, haemorrhage, antibacterial infection, dysuria, fever, hyperthyroidism and ulcer, Possess ntitumor, antifertility and abortifacient properties,	0.32
10	*Annona squamosa* Linn	Beneficial for cardiac disease, hyperthroidism and cancer, Used as a purgative and applied on ulcer and wounds. Possess antitumor, antidiabetic, antimicrobial, antihyperlipidemic and hepatoprotective activity.	0.30
11	*Uvaria lurida* Hk. f. & T.	Treatment of inflammatory disease, rheumatism, acute allergic reaction, inflammatory liver disease and swollen joints.	0.19
12	*Litsæa glutinosa* (Lour.)C.B.Rob.	Treatment of furnacle, traumatic injury, reducing swelling and soreness.	0.16
13	*Litsæa polyantha* Juss.	Treatment of fractures and dislocation. Cures gonorrhea, skin disease, boil, diarrohea, pain and bruises	0.24
14	**Family: Rutaceae** *Glycosmis pentaphylla* Correa.	Treatment of bilious complaints, cough, jaundice, fever, inflammation, rheumatism, anaemia, vermifuge. Also known for its hepatoprotective, antiinflammatory, antimicrobial, antiulceritive, chemoprotective,	0.37

		antipyretic, antitumor, wound healing and insecticidal activity.	
15	*Toddalia asiatica* Lamk.	Treatment of sprains, convulsions, intercostal neuralgia, cough, malaria, garstral dysentery, snakebite and furnucle. Possess antiviral, antimalarial and anticancer effect.	0.27
16	*Murraya koenigii* Spreng.	A number of pharmacological activities such as anti-tumor, anti-oxidative, anti-mutagenic and anti-inflammatory have been reported	0.27
17	*Aegle marmelos* Correa.	Known to lower blood glucose levels. Plant also possesses antifungal properties	0.22
18	*Zanthoxylum acanthopodium*	Treatment of fever, gastric problems, liver complaints and dental problems. Possess antihelminthic and carminative properties.	0.18
19	*Clausena excavata* Burm.	Treatment of nausea, constipation and cardio-vascular disorders, Also possess anti-inflammatory and spasmolytic properties.	0.29
20	*Citrus medica* Linn.	Acts as astringent and to treat blood disorders. Also possess analgesic, antidiabetic, antitumor, antimicrobial and hypocholesterolamic properties.	0.44
21	*Citrus grandis* (L.) Osbeck	Possess antioxidant, antimicrobial and antidiabetic properties. Effective as a cardiac stimulant and stomach tonic.	0.32
22	*Citrus limon* (L.) Burm. f.	Provides relief from cough and cold, fever, soreness, rheumatism, kidney disorders, cardiac diseases, anaemia and digestive problems	0.23
23	*Citrus aurantium* Linn.	Effective against dyspepsia, asthma, obesity, sleeplessness	0.38
24	**Family: Aristolochiaceae** *Aristolochia tagala* Cham. & Schlect.	Effective against stomachache, snakebite ,dental problems and rheumatism	0.36
25	*Aristolochia saccata* Wall.	Provides relief from fever, diarrohea and dysentry	0.23
26	*Aristolochia indica* L.	Effective against cholera, fever, bowel problems, ulcers, leprosy, and skin diseases. Also being employed as an abortifacient and antineoplastic	0.47

Discussion:

Investigation on papilioninae has been conducted with the aim to explore the wide repertoire of medicinal botanicals satisfying their larval food resource requirements. Such strategies designed to effectively utilize these plants thereby enlightens the significance of these plants in life of butterflies. Additionally phytochemical and pharmacological properties of such plants have dominated most of the primary treatment imparted in healthcare system, Significantly, Rutaceae, Lauraceae, Annonaceae, Magnoliaceae and Aristolochiaceae known for their secondary metabolites constituents are beneficial not only for papilioninae but also for the folklore therapists.

Terpenoids (viz. α-pinene, β-pinene, limonene, myrcene, linalool, terpinolene) are abundantly associated with *Citrus* [21-22]. Besides hesperidin and neohesperidin found both in *C.limon* and *C. grandis*, naringenin, naringin and rutin are predominant flavonoids isolated from *C. grandis* [23]. *Z. acanthopodium* is recognised for its unique terpenoids (ie. linalool, phytol, 1-8 cineole, farnesol, β-caryophyllene) [24]. *Toddalia*, also possesses alkaloids viz. toddalin, toddalidimerine, dihydroviridine along with terpenoids [25]. Alkaloids such as aeglin, aegelenine, dictamine, fragrine, [26] along with few terpenoids (p-cymene, limonene and α-phellandrene) [26-27] are essential ingredients of *A. marmelos.* Koenine, koenigine, girininbine, koenimbine, isomahanine, bismahanine are reported from *M. koenigii* [28]. Besides terpenoids (eudesmol, pinene, camphene, limonene, caryophyllene), some alkaloids viz. magnocurarine, magnoflorine, auonaine, michelarbine, liriodenine, salicifol, tubocurarine are found in *Magnolia* [29]. Additionally *Polyalthia* are known for its two clerodane type of diterpenes[30]. Besides, oxygenated mono and sesquiterpenes, mono and sesquiterpene hydrocarbons are found among *Persea* [31]. Tetradecanal, tridecanal, myristic acid from *L. polyantha* bark [32] and 9,12 octadecadienoic acid, hexadecanoic acid, stigmasic-5-en-3ol from *L. glutinosa* [33] also reveal some interesting findings. Terpenoids from *C. camphora* include cineol, limonene, α-humulene, β-cardinine, D-camphor, α-terpineol, linalool,γ-terpinene, α-pinene, β-pinene and eugenol [34-36]. *C. tamala* bark has also reported the occurrence of following terpenoids viz. phellandrene, eugenol, linalool, α-pinene, p-cymene, β-pinene and limonene [37]. Both alkaloids (ie. phenolic and non-phenolic alkaloids, aporphine alkaloids: annonamine, crystalline alkaloid: muricine and muricinine, roemerine, corydine, norisocorydine, samoquasine A) and terpenoids (linalool, carvone, acetogenin: squamocin A, squamocin B are also abundant in *Annona* [38-39]. 8 types of aristolactam type alkaloid and 7 arristolochic acid derivatives are quite significant in *A. tagala* [40] However, terpenoids viz. pinene, pinocarvone, trans-pinocarvol, are the major components of *A. indica* [41].

A cocktail of terpenes (α-pinene, sabinene, β-myrcene, limonene, β- phellandrene, ocimene, germacrene-A, bisabolene, germacrene-B, oxygenated sesquiterpenes and 3-hydroxy-2-butyrate) released by fourth instar larva along with aliphatic acids and their esters in fifth instar are worth mentioning. Graphini (*G. doson* and *G.antiphates*) are known to possess aliphatic acids and their esters in both the final instar stages [42]. Papilionii exudates reveal presence of terpenoids in fourth instar followed by aliphatic acids in final instar stage [43-45]. In contrast troidinii's osmeterial secretion contains aristolochic acids (AAs) [46-48]. Caterpillars of *P.polytes, P. demoleus, P. paris* were reported to experience a change in body colour and pattern during transition from penultimate to ultimate stage. Such an unique ability to mimic brown and white bird droppings to small green snakes [49] in fifth instar could be attributed to an alteration in their osmeterial chemistry [50]. Therefore such variability in the osmeterial composition among Papilionini and Graphinii provide a clue towards plesiomorphic trait in them [48]. On the contrary troidinii upon sequestering AAs are know to generate a primary line of defence against birds and vertebrates [51].

Greater utilization values (U_v) of *A. indica* and *C. medica* in this study highlighted their probable benefits for the proponents of traditional medicine. Alkaloids, widely recognised for their immense therapeutic benefits viz. antitumor, anti-inflammatory, antimalarial and antiviral contribute towards several modern day drug formulation [52-54]. Equally beneficial and enormously popular, plant terpenoids are considered to be a proven remedy against fever, bronchitis, asthma, microbial, cancer, fungal and viral infection [55,37,56,22, 29].

Episodes of *G. cloanthus, G. sarpedon, G. eurupylus, G. doson* and *G agamemnon* feeding from more than one plant family describing their polyphagous nature is probably aimed at utilizing a wide spectrum of secondary metabolites Papilio demoleus and P.polytes feeding on multiple species of rutaceous plants also denote their polyphagous nature Thus polyphagous species are known to employ a wide repertoire of phytochemicals probably as stimulus for oviposition. In contrast strict monophagy as evident among *G.antiphates, G. nomius, A. polyeuctus, A. aidoneus, T. helenus* signified their restricted feeding preference. Interestingly, such single family association among papilioninae viz.papilionii with Rutaceae and troidini with Aristolochiaceae are noteworthy [57-58].

Conclusion:

Ethnobotany serves as a tool for preparation of several novel herbal formulations by utilization of the indigenous knowledge of ethnic people. Custom associated with the use of wild population of medicinal plants has lead to the creation of enormous pressure leading to uncontrolled exploitation of forest resource for commercial purposes. Significantly such mismanagement of floral resources could negatively effect the larval population of butterflies dependant on those specific medicinal plants. Thereby proper conservation and management of medicinal plants becomes an important issue in the present scenario. Thus the present study could provide a platform for designing of futuristic policies regarding natural resource management keeping in mind the demand of traditional practitioners. Additionally such a system should also ensure the availability of larval food resource of butterflies in turn ensuring their survival.

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