Journal of Molecular Structure 1249 (2022) 131528

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstr

# An efficient Brønsted acid ionic liquid catalyzed synthesis of novel spiro1,2,4-triazolidine-5-thiones and their photoluminescence study

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ARTICLE INFO

*Article history:*
Received 1 June 2021
Revised 6 September 2021
Accepted 17 September 2021
Available online 20 September 2021

*Keywords:*
11H-Indeno[1,2-b]quinoxalin-2-one
Thiosemicarbazide
Ionic liquid
Spiro 1,2,4-triazolidine-5-thione
Bronsted acid.

ABSTRACT

We have synthesized a novel Bronsted acidic ionic liquid, 1-(2-hydroxyethyl)-1-(4-sulfobutyl)piperidin-1-ium hydrogen sulfate, $[HEPiPYBSA]^+HSO_4^-$ and explored its catalytic efficiency for synthesis of indenoquinoxalone tethered spiro-1,2,4-triazolidine-5-thiones from reaction of 11H-[1,2-b]quinoxalin-11-one and thiosemicarbazide. The most stable geometries of synthesized ionic liquid (IL) $[HEPiPYBSA]^+$ $HSO_4^-$ were obtained through systematically optimization by the DFT theory at B3LYP/6-31G* level. A photoluminescence study of the synthesized spiro-1,2,4-triazolidine-5-thiones revealed a remarkable fluorescent activity. The advantages of the present method are a reusable hydrophilic green catalyst, mild reaction conditions, use of benign solvent system, short reaction span, high atom economy and wide substrate scope.



## 1. Introduction

Nowadays, the development of environmentally friendly synthetic procedures became a major concern in chemical industry, due to continuing depletion of natural resources and growing awareness [1–6]. One of the major efforts in modern academic research is to replace the environmentally damaging organic solvents, especially those which are volatile and difficult to comprise. Most notably, ionic liquids (ILs) have attracted considerable interest as environmentally benign reaction media because of their fascinating and intriguing properties [7–12]. They offer an alternative and ecologically sound medium compared to the conventional organic solvents due to their negligible vapor pressure, ease of handling and potential for recycling. Moreover, their high compatibility with transition metal catalysts and limited miscibility with common solvents, enables easy product and catalyst separation with the retention of the stabilized catalyst in the ionic phase [13,14].

The heterocyclic moieties are important skeleton of long range of molecules involving pharmaceutical drugs, polymers, biological active structures and natural products. The varied class of nitrogen-containing heterocycles includes abroad fraction of organic products, many of which have found significant applications in agrochemistry, material science and medicinal chemistry. Thus, there is continuing attention in the expansion of an expeditious, atom-economic, and environmentally benign synthetic procedures for the preparation of N-heterocyclic compounds [15–22].

The quinoxaline rings are frequently found in a broad spectrum of potential bioactive agents and natural products [23] and also act as diverse precursors in organic synthesis [24]. Considering the synthetic and practical applications of quinoxaline molecules, numerous tactics for the preparation of this scaffold have been explored [25–28]. The indenoquinoxaline moieties are well recognized pharmacophore as it also possesses anticancer [29], anti-inflammatory [30], antitumor [31] activity. The Schiff base derivatives of indenoquinoxalines are well known antiviral agents and are cytotoxic in nature [32]. Their Oximes are noncytotoxic inhibitors of inflammatory cytokine [33].

The synthesis of spiroheterocycles is a privileged interest of synthetic chemists as they are key moieties in many natural products and pharmaceutical compounds [34–36]. Compounds with spirocyclic structure having one common $sp^3$ carbon atom between two rings an interesting synthetic challenge due to their important structural rigidity and complexity [37,38]. Spiro heterocycles containing nitrogen, oxygen, and sulfur atom have shown a notable role in biological processes and have exhibited significant pharm-

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https://doi.org/10.1016/j.molstruc.2021.131528
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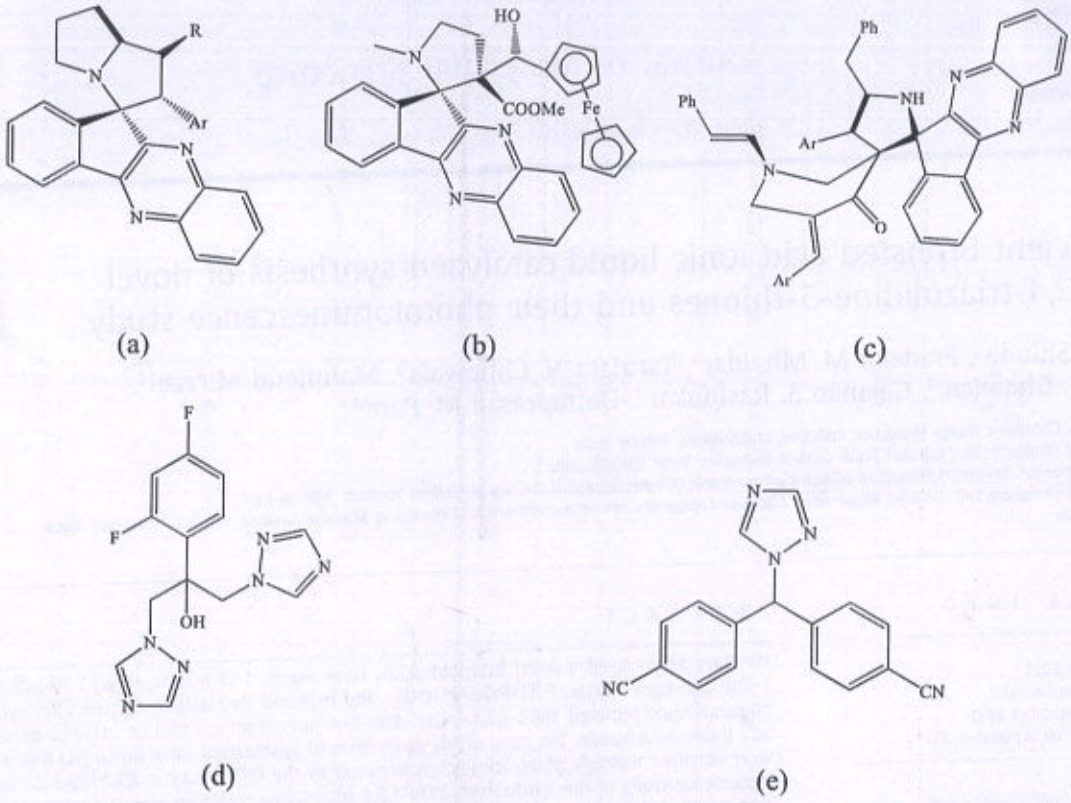
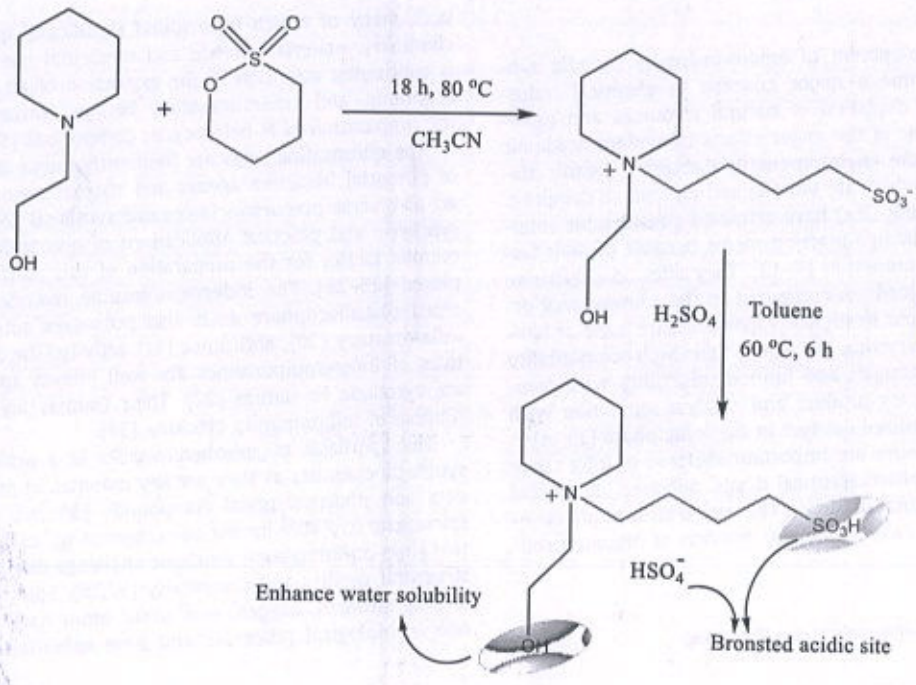
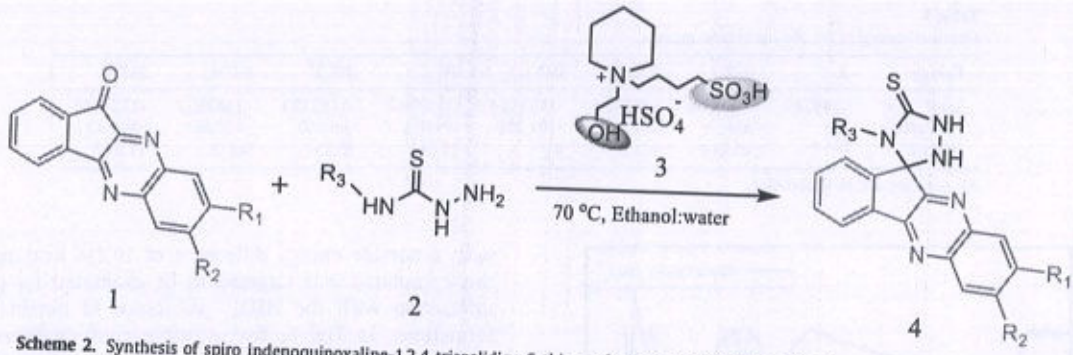



**Fig. 1.** Representative structures of biologically active spiro indenoquinoxalines and 1,2,4-triazole derivatives.




**Scheme 1.** Synthesis of Brønsted acid ionic liquid, $[HEPiPYBSA]^+ HSO_4^-$.




**Scheme 2.** Synthesis of spiro indenoquinoxaline-1,2,4-triazolidine-5-thiones from 11H-[1,2-b]quinoxalin-11-ones and thiosemicarbazide

macological activities [39]. The spiro derivatives of indenoquinoxalines exhibit potent AChE inhibitory activity, anticancer, (Fig. 1a) antibacterial, (Fig. 1b), antimicrobial, (Fig. 1c), antioxidant and antitubercular properties [40].

The nitrogen-containing five member heterocycle scaffolds are key building blocks of biologically important molecules as they play vital role in their essential physiological processes. The compounds with 1,2,4-triazole skeleton possess a broad pharmacological activities viz. antibacterial [41], antifungal [42], (Fig. 1d) anthelmintic [43], analgesic, cyclo-oxygenase inhibitor [44], anticancer, [45] (Fig. 1e) anticonvulsant, [46] antioxidant, anti-malarial as well as anticipated activity [47].

Our interest in synthesis of heterocyclic compounds particularly, spiroheterocycles resulted in investigation of several routes for synthesis of spiroheterocycles. Initially, we reported two catalyst-free multi-component synthesis of novel spiropyranopyrazole derivatives from pyrazolone, isatin and malononitrile [48,49]. A glycine nitrate catalyzed eco-benign method for synthesis of novel spiro-1,2,4-triazolidinones from isatin and semicarbazide/thiosemicarbazide in water has also been reported [50].

Construction of hybrid of two active pharmacophores has been considered as better approach to achieve efficiently biological active targets. Heterocycles having quinoxaline and 1,2,4-triazole scaffolds are key structural motif in organic synthesis. While the quinaxolines and 1,2,4-triazole have attracted remarkable attention in generation of new drugs but building of compounds incorporating both bioactive motifs in a single molecular framework through spiro carbon has not been reported so far.

As a part of our investigation in the application of novel ionic liquids in synthesis of spiroheterocycles [51], herein, we report expeditious method for the synthesis of spiro-1,2,4-triazolidine-5-thiones from indenoquinoxalone and thiosemicarbazide employing catalytic amount of 1-(2-hydroxyethyl)-1-(4-sulfobutyl)piperidin-1-ium hydrogen sulfate, $[HEPiPYBSA]^+HSO_4^-$ Brønsted acidic ionic liquid catalyst (Scheme 2).

## 2. Result and discussion

Initially, we designed and focused our attention towards synthesis of task-specific hydrophilic ionic liquid (TSIL), $[HEPiPYBSA]^+HSO_4^-$ which could facilitate synthesis of spiro1,2,4-triazolidine-5-thiones. The hydrogen sulfate group in ionic liquid favors the synthesis of triazole through protonation. The hydrophilic nature of hydroxy group leads formation of hydrogen bonds and provides easy association of reactants with solvent molecules resulting smooth reaction. The outline for synthesis of $[HEPiPYBSA]^+HSO_4^-$ is highlighted in Scheme 1.

$[HEPiPYBSA]^+HSO_4^-$ is a Brönsted acidic ionic liquid with acidic hydrogen in functional group and on an anion synthesized through well-known sultone method [52,53]. Reaction of the neutral nucleophiles 1,(2-hydroxyethyl) piperidine with 1,4-butane sultone produced the requisite zwitterion in good yield. The zwitterion possessed an alkane sulfonate group covalently tethered to the nitrogen of piperidine. In the second step, the zwitterion acidification is accomplished by combining 1:1 molar quantities of the zwitterion with sulfuric acid to convert the pendant sulfonate group into an alkane sulfonic acid. Overall the IL, $[HEPiPYBSA]^+HSO_4^-$ formed via transformation of the zwitterion into an IL cation bearing an appended sulfonic acid group, with the conjugate base of the sulfuric acid (hydrogen sulphate) resulting formation of an anion of IL. After successful synthesis of ionic liquid, [HEPiPYBSA]+ HSO4- we confirmed its formation by $^1H$, $^{13}C$ NMR, IR, and MS.

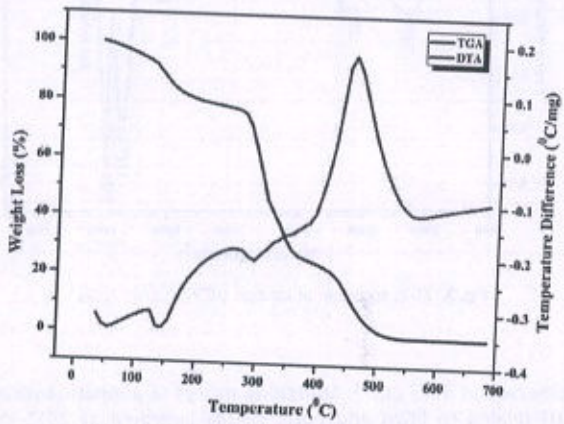



**Fig. 2.** TGA-DTA analysis of $[HEPiPYBSA]^+ HSO_4^-$.

The thermal stability of $[HEPiPYBSA]^+ HSO_4^-$, IL was studied by thermogravimetric analysis (TGA) and differential thermal analysis (DTA). The TGA was recorded at 25 to 700°C in air atmosphere with temperature increment at 10°C/min (Fig. 2.) The initial weight loss about 7.38% upto 125 °C in TGA and slight endotherm at 52.90 °C in DTA are due to moisture or loosely bounded water molecules in the sample. The second weight loss from 125-275 °C (16.63%) in TGA and deep endotherm at 141.19 °C are associated with disintegration of acidic anion part. Weight loss at 275-370 °C (48.22%) in TGA and deep endothermic event at 301.0 °C in DTA are due to the decomposition of cationic chain containing –OH and $-SO_3H$ groups and loss organic moiety. The final weight loss beyond 400 °C (27.78%) was observed due to degradation of carbonaceous matter by removal of $CO_2$ and $O_2$ gaseous molecules. Overall the TGA-DTA profile revealed that the catalyst is thermally stable upto 375 °C.

The FT-IR analysis was carried out to confirm the chemical structure of $[HEPiPYBSA]^+ HSO_4^-$(Fig. 3). The most significant characteristic broad absorption band for –OH stretching vibrations

**Table 1**
Obtained energies for the optimized models.

| Energy | IL-1 | IL-2 | HSO4⁻ | INT-1 | INT-2 | INT-3 | INT-4 | INT-5 |
|---|---|---|---|---|---|---|---|---|
| Total | -744349 | -744339 | -438704 | -1183154 | -1183142 | -1183139 | -1183153 | -1183142 |
| Interaction | n/a | n/a | n/a | -101.259 | -89.618 | -86.002 | -100.881 | -89.392 |
| Delta-E | 0 | 10.214 | n/a | 0 | 11.641 | 15.257 | 0.378 | 11.867 |

All energies are in Kcal.mol$^{-1}$.

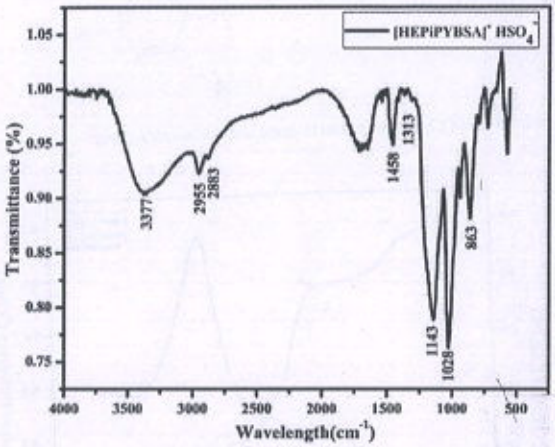



**Fig. 3.** FT-IR spectrum of catalyst [HEPiPYBSA]$^+$ $HSO_4^-$.

is observed at 3377 cm$^{-1}$. Stretching modes of aliphatic hydrogens (-CH) related to ethyl and butyl chains appeared at 2955 cm$^{-1}$. However, the peaks at 1313 and 1458 cm$^{-1}$indicate the bending vibrations of methylene groups (-$CH_2$). Furthermore, the symmetric and asymmetric stretching vibrations for S=O groups observed at 1143 and 1028 cm$^{-1}$ [54].

### *2.1. Density-functional theory (DFT) study: A theoretical analysis of [HEPiPYBSA]$^+$ $HSO_4^-$ conformations*

To theoretically recognize possible confirmations of [HEPiPYBSA]$^+$ $HSO_4^-$ IL, the B3LYP/6-31G* density functional theory (DFT) calculations were performed using the Gaussian program as benefit of computer-based works for solving problems in chemistry [55–57]. To this aim, two stable conformations of IL were recognized according to the performed optimization to interact with the $HSO_4^-$ substance (Fig. 4). As indicated by the evaluated energies (Table 1), IL-1 was found more stable than IL-2 with a notable energy difference of 10.214 Kcal.mol-1. Therefore, this compound was targeted to be examined for participating in interaction with the $HSO_4^-$ substance to obtain the final conformations. In Fig. 5, five possible conformations are obtained by performing optimization processes were found with different stabilities and interaction energies as indicated by the obtained values of Table 1. In these INT-1 to INT-5 conformations, all models were seen achievable regarding the values of total energies and negative values of interaction energies. To find values of interaction energies, differences of total energies between the final model and components were measured. In such case, values of delta were obtained by difference of interaction energies between the final conformations and the most stable model. As a consequence, the idea of such interacting situation was affirmed regarding the evaluated structural conformations and their corresponding energies. The strength of interacting models were ranged in this order: INT-1 > INT-4 > INT-2 > INT-5 > INT-3. All optimized geometries were listed in a supplementary file.

The structural confirmation and existence of anion as $HSO_4^-$ was described by dissociation constant of sulphuric acid. Kolthoff et al. determined the first and second dissociation constant of sulphuric acid in aprotic protophobic and aprotic protophylic solvents [58,59]. The second dissociation constant of sulphuric acid is greater, which ensures that the stability of $HSO_4^-$ conjugate base of sulphuric acid is more stable than $SO_4^{2-}$, in fact $HSO_4^-$ will not further dissociates to $SO_4^{2-}$ in toluene.

After successful synthesis and characterization and structural confirmation of Brønsted acid ionic liquid, [HEPiPYBSA]$^+$ $HSO_4^-$ we explored its catalytic application in organic synthesis. In continuation with our interest in investigating novel class of triazoles, herein we explored catalytic activity of synthesizeed IL for synthesis of novel spiro 1,2,4-triazolidine-5-thiones using indenoquinoxalone and thiosemicarbazides.

Initially, we focused our attention on the optimization of suitable solvents for model reaction of 11H-indeno[1,2-b]quinoxalin-11-one and thiosemicarbazide employing [HEPiPYBSA]$^+$ $HSO_4^-$ (20 mol %) as a catalyst (Table 2) under reflux condition. Polar aprotic solvents such as $CH_3CN$, DCM, DMF, THF were tested for model reaction which resulted in low yield of 1,2,4-triazolidine-5-thiones

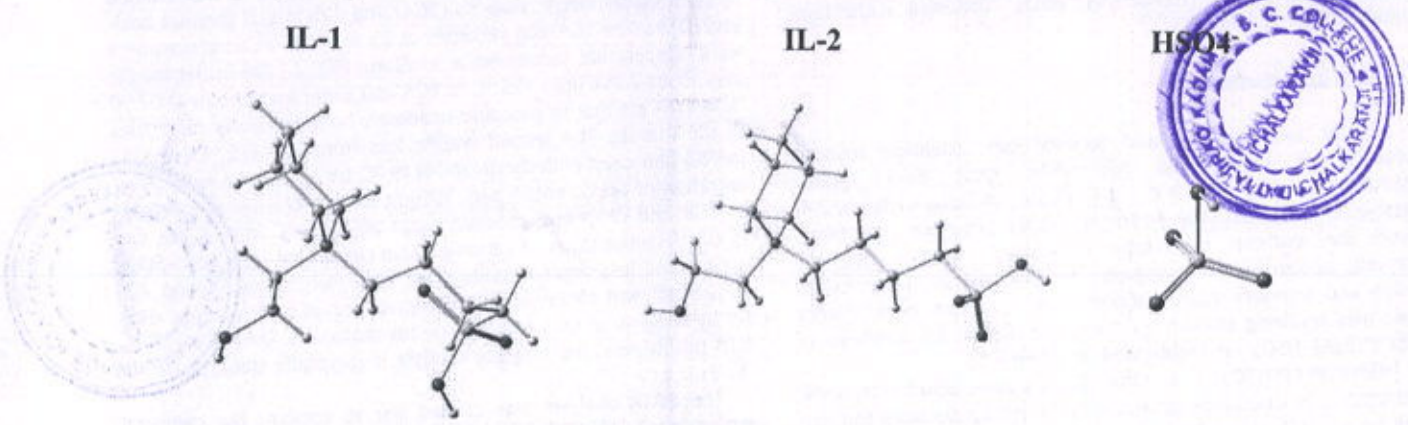



**Fig. 4.** The optimized models of two conformations of [HEPiPYBSA]$^+$ and HSO4$^-$.

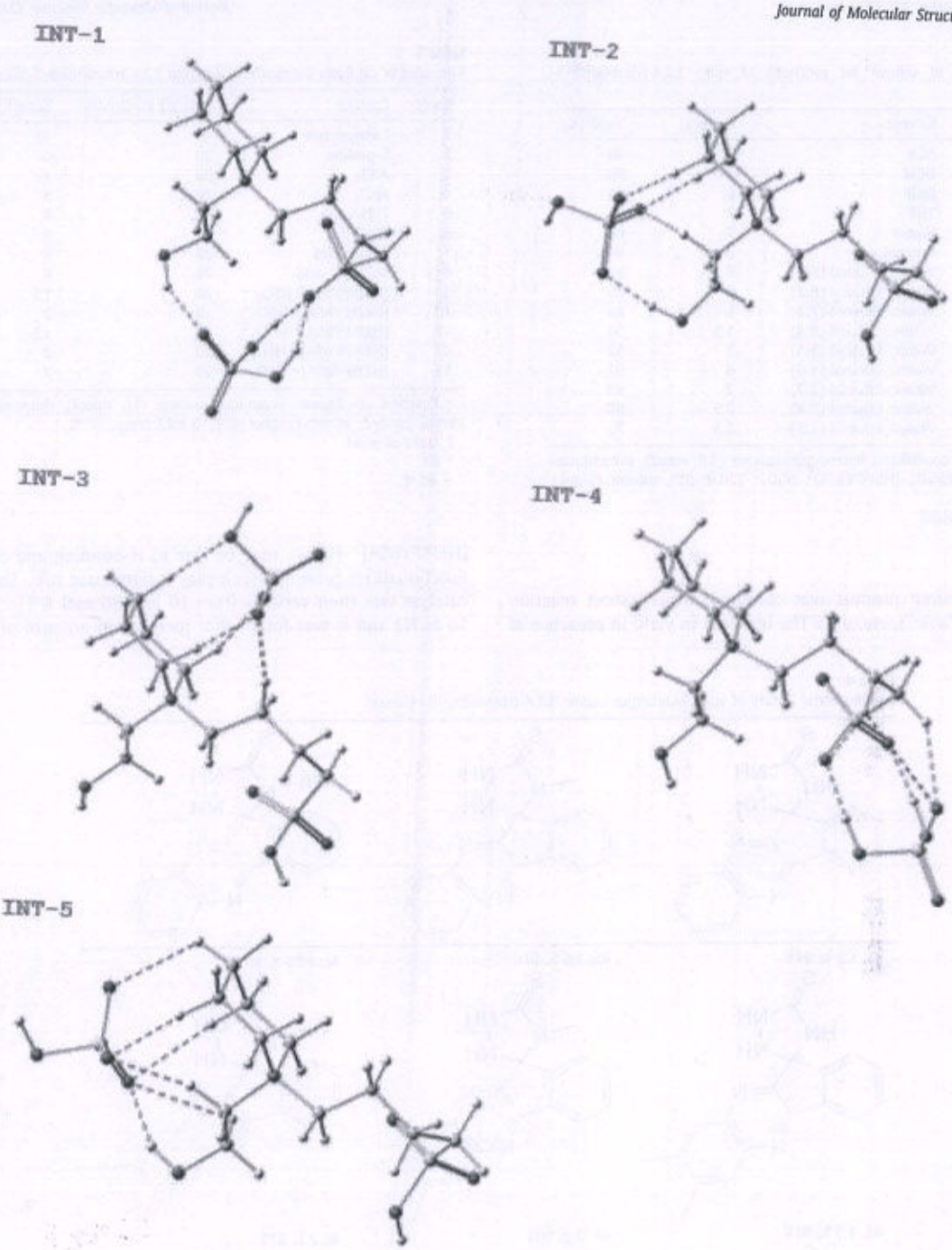



**Fig. 5.** The optimized models of interacting conformations of IL-1 and $HSO_4^-$.

(Table 2, entries 1-4). The use of water resulted in 64 % yield due to scanty solubility of reactants (Table 2, entry 5). Interestingly, in ethanol 74% yield of product was obtained in 3 h (Table 2, entry 6). In light of these results and our earlier experience in use of mixed solvent system [60], we decided to employ mixed solvent system, water: ethanol for the reaction (Table 2, entries 7-15). We obtained best result in terms of yield and reaction time in water: ethanol (6:4 v/v) (Table 2, entry 10).

The screening of catalysts for model reaction was examined (Table 3). The uncatalysed reaction leads to only 38% yield of product even under reflux condition (Table 3, entry 1). While the amino acid as a catalyst, L-proline also exhibited moderate yield of the product even after 6 h (Table 3, entry 2). In presence of 20 mol % of different Lewis acid catalysts like $AlCl_3$, $FeCl_3$, p-TSA, and EPZ 10, no significant results are obtained (Table 3, entries 3-6). The other catalysts like acetic acid and sulfamic acid failed to give high yield for present transformation even after long reaction time (Table 3, entries 7-8). These results provoked us to employ synthesized task specific ionic liquid, $[HEPiPYBSA]^+ HSO_4^-$. Interestingly, with 20 mol % of $[HEPiPYBSA]^+ HSO_4^-$ as a catalyst 94%

**Table 2**
Optimization of solvent for synthesis of spiro 1,2,4-triazolidine-5-thiones[a].

| Entry | Solvent | Time(h) | Yield[b](%) |
|---|---|---|---|
| 1 | ACN | 5 | 46 |
| 2 | DCM | 5 | 39 |
| 3 | DMF | 6 | 41 |
| 4 | THF | 8 | 37 |
| 5 | Water | 7 | 64 |
| 6 | Ethanol | 3 | 74 |
| 7 | Water: Ethanol (9:1) | 6 | 84 |
| 8 | Water: Ethanol (8:2) | 6 | 85 |
| 9 | Water: Ethanol (7:3) | 6 | 88 |
| 10 | Water: Ethanol (6:4) | 1.5 | 94 |
| 11 | Water: Ethanol (5:5) | 3 | 92 |
| 12 | Water: Ethanol (4:6) | 4 | 91 |
| 13 | Water: Ethanol (3:7) | 2 | 90 |
| 14 | Water: Ethanol (2:8) | 2.5 | 86 |
| 15 | Water: Ethanol (1:9) | 2.5 | 76 |

[a] Reaction conditions: Indenoquinoxalione (1.0 mmol), thiosemicarbazide (1.0 mmol), [HEPiPYBSA]$^+$ $HSO_4^-$ (20 mol%), solvent (5 mL), temp.: 70 °C
[b] Isolated yield.

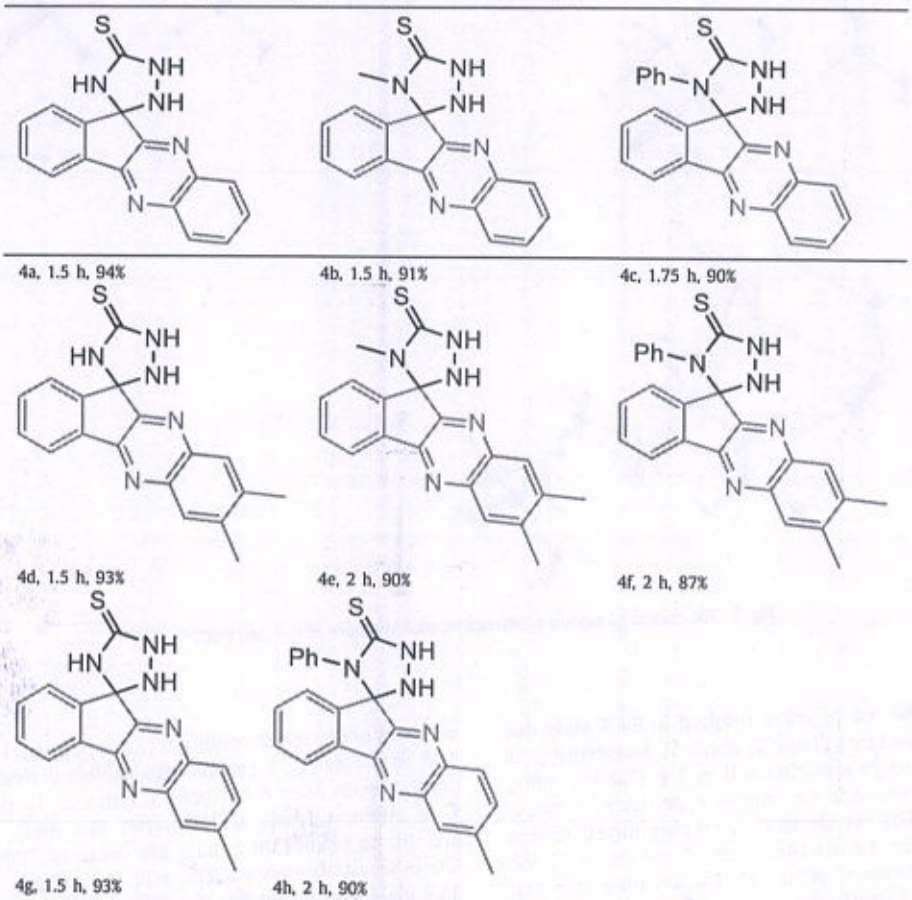
**Table 3**
Screening of catalysts for synthesis of spiro 1,2,4-triazolidine-5-thiones[a].

| Entry | Catalyst | Catalyst load(mol%) | Time(h) | Yield[b](%) |
|---|---|---|---|---|
| 1 | Catalyst free | - | 12 | 38 |
| 2 | L-proline | 20 | 6 | 65 |
| 3 | $AlCl_3$ | 20 | 4 | 60 |
| 4 | $FeCl_3$ | 20 | 5 | 65 |
| 5 | P-TSA | 20 | 6 | 72 |
| 6 | EPZ 10 | 20 | 6 | 40 |
| 7 | Acetic acid | 20 | 8 | 56 |
| 8 | Sulfamic acid | 20 | 4 | 79 |
| 9 | [HEPiPYBSA]$^+$$HSO_4^-$ | 20 | 1.5 | 94 |
| 10 | [HEPiPYBSA]$^+$ $HSO_4^-$ | 10 | 2 | 86 |
| 11 | [HEPiPYBSA]$^+$ $HSO_4^-$ | 30 | 1.5 | 93 |
| 12 | [HEPiPYBSA]$^+$ $HSO_4^-$ | 20 | 2 | 48[c] |
| 13 | [HEPiPYBSA]$^+$ $HSO_4^-$ | 20 | 2 | 92[d] |

[a] Reaction conditions: indenoquinoxalone (1.0 mmol), thiosemicarbazide (1.0 mmol), catalyst, Water: Ethanol (6:4) (5 mL), temp.: 70°C
[b] Isolated yield
[c] RT
[d] 80 °C.

yield of the desired product was obtained in very short reaction time at 70 °C (Table 3, entry 9). The increase in yield in presence of [HEPiPYBSA]$^+$ $HSO_4^-$ may be due to H-bonding and co-ordination functionalities present which play a significant role. The amount of catalyst was then verified from 10 and 30 mol % (Table 3, entries 10 & 11) and it was found that increase in amount of catalyst did

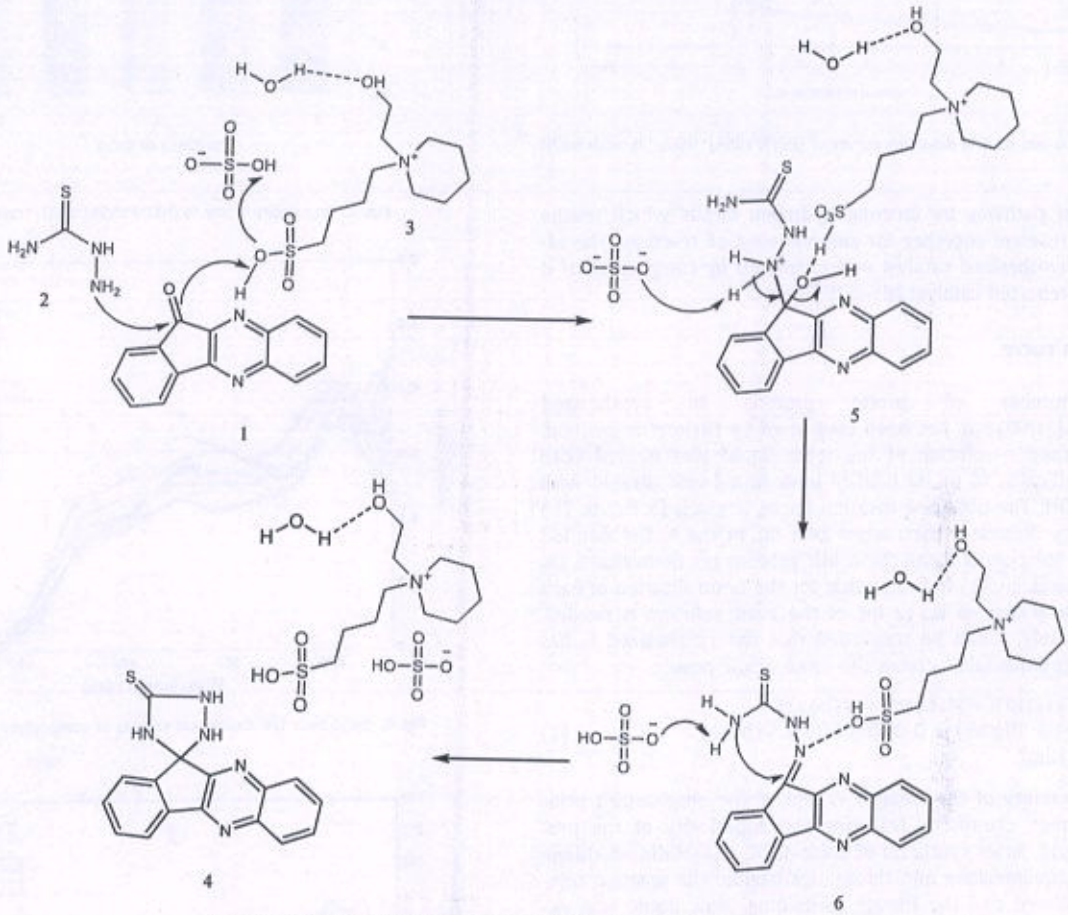
**Table 4**
Combinatorial library of spiro indenoquinoxaline-1,2,4-triazolidine-5-thiones[a].




[a] Reaction conditions: indenoquinoxalone (1mmol), thiosemicarbazides (1mmol), water:ethanol(5 mL), [HEPiPYBSA]$^+$$HSO_4^-$IL (20 mol %), temp = 70°C

**Table 5**
Comparison study of $[HEPiPYBSA]^+ HSO_4^-$.

| Entry | Catalyst | Reaction Condition | Time | Yield (%) | Ref |
|---|---|---|---|---|---|
| 1 | $[PySOPy][HSO_4]_2$ | EtOH, RT | 1 h | 55 | 61 |
| 2 | Gly-$NO_3$ | $H_2O$, 80 °C | 3 h | 83 | 62 |
| 3 | Catalyst free | PEG-400, 80 °C | 8 min | 86 | 63 |
| 4 | $[HEPiPYBSA]^+ HSO_4^-$ | Water: EtOH, 70 °C | 1.5 h | 94 | This work |



**Scheme 3.** Plausible mechanism for synthesis of spiro 1,2,4-triazolidine-5-thiones.

not affect the yield of product and reaction time. At room temperature the yield of product was only 48% (Table 3, entry 12). No significant change in yield of product was observed when reaction was performed at 80 °C (Table 3, entry 13). Hence, the optimized conditions of the reaction are use of 20 mol % of $[HEPiPYBSA]^+ HSO_4^-$ in water:ethanol (6:4) at 70 °C.

Under the optimized reaction conditions the generality of the developed method with various substituted indenoquinoxalone and thiosemicarbazide/substituted thiosemicarbazides were carried out (Table 4, entries 4a-4h). Unsubstituted indenoquinozalone react smoothly with thiosemicarbazides, N-methyl thiosemicarbazide and N-phenylthiosemicarbazide in short reaction time with high yield of products (Table 4, entries 4a-4c). Substituted Indenoquinoxalone also afford corresponding spiro-1,2,4,-triazolidine-5-thiones in significant yield in short reaction time (Table 4, entries 4d-4h). Synthesized derivatives are confirmed by spectral techniques viz. IR, $^1H$ NMR and LCMS analysis. The reaction of 11H-[1,2-b]quinoxalin-11-one and thiosemicarbazide offered spiro[indeno[1,2-b]quinoxaline-11,3′-[1,2,4]triazolidine]-5′-thione in excellent yield (Table 4, entry 4a). In the IR spectrum of the desired product, the band at 1496 $cm^{-1}$ confirms the presence of amidic thiocarbonyl group. $^1H$ NMR spectrum depicts three $D_2O$ exchangeable singlets at $\delta$12.60, 9.04 and 8.81 ppm for three –NH protons of triazole ring. Aromatic protons of quinoxaline ring were observed at $\delta$ 7.64 to 8.18. In mass spectrum, the characteristic peak at m/z 306 confirms the formations of spiro[indeno[1,2-b]quinoxaline-11,3′-[1,2,4]triazolidine]-5′-thione.

The successful synthesis of diversely substituted spiro 1,2,4-triazolidine-5-thiones encouraged us to explore mechanism of the formation of product. The plausible mechanism is depicted in Scheme 3. Initially, the acidic functionality of $[HEPiPYBSA]^+HSO_4^-$ IL (3) enhance electrophilic character of carbonyl group of indenoquinoxalione (1) by protonation which facilitate nucleophilic attack of $-NH_2$ of thiosemicarbazide (2) leading to formation of adduct 5. The dehydration of adduct 5 leads to the formation of Schiff's base (6). Finally, the subsequent intramolecular nucleophilic attack of thioamidic $-NH_2$ of thiosemicarbazide on electron deficient carbon furnished the desired product 4. From the structure and nature of Bronsted acid $[HEPiPYBSA]^+HSO_4^-$ it has been hypothesized that the hydrogen bonding nature of hydroxyl groups assists the cou-

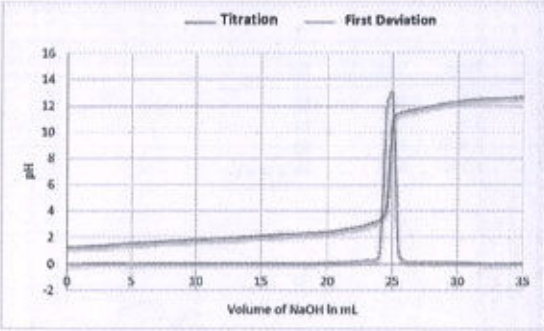



Fig. 6. Titration and its first deviation curves of $[HEPiPYBSA]^+HSO_4^-$ IL with NaOH

tire reaction pathway by forming hydrogen bonds which results binding of reactant together for smoothening of reaction. The efficiency of synthesized catalyst was examined by comparison of it with other reported catalyst [61–63] (Table 5).

## 3. Titration curve

The number of protic protons in synthesized $[HEPiPYBSA]^+HSO_4^-$ IL has been confirmed by titrimetric method. In this method a solution of the ionic liquid was treated with NaOH. Specifically, 10 mL of 0.06 M ionic liquid was titrated with 0.05 M NaOH. The obtained titration curve is given in Fig. 6. The figure clearly illustrates that, when 24.5 mL of the NaOH solution is added to solution of IL, all the acidic protons get neutralized. On the other hand, Eq. (1) indicates that for the neutralization of each of the acidic proton of IL, 12 mL of the basic solution is needed. From this study, it can be concluded that the synthesized IL has two protic protons with almost the same acidic power.

$$\begin{aligned} &M(acid) \times V(acid) = M(base) \times V(base) \\ &0.06(molar) \times 10(mL) = 0.05(molar) \times V(base) \\ &V(base) = 12mL \end{aligned} \quad (1)$$

The reusability of the catalyst is one of the emphasized principles of green chemistry, featuring the superiority of the proposed method. After synthesis of spiro-1,2,4, triazolidine-5-thione from indenoquinoxalone and thiosemicarbazide, the reaction mixture was filtered and the filtrate containing ionic liquid was extracted with ethyl acetate to remove any soluble impurities. The aqueous layer was separated and evaporated under pressure to recover the ionic liquid in pure form. The recovered catalyst was then employed to the same model reaction for next catalytic cycle and examined upto five cycles. The reusability study of $[HEPiPYBSA]^+HSO_4^-$ is depicted in Fig. 7. The catalyst displayed efficient activity up to five reaction cycle without notable change in the yield of the products.

### *3.1. Photoluminescence properties of synthesized spiroindeno-quinoxaline-1,2,4-triazolidine-5-thiones*

The absorption and fluorescent spectra of spiro indeno-quinoxaline-1,2,4-triazolidine-5-thione were examined and depicted in Figs. 8 & 9. Among all available solvents, dimethyl sulphoxide (DMSO) was best solvent for absorption study due to excellent solubility of all compounds. Photophysical properties of organic molecules are influenced by presence of electron donating and withdrawing groups on it. The solvent polarity also affect to some extent. The electron donating groups on molecule favors the extended $\pi$-conjugation facilitating higher HOMO and lowers the HOMO-LUMO gap which results in absorption at higher wavelength (red-shift). However, the distracted fluorescence emission

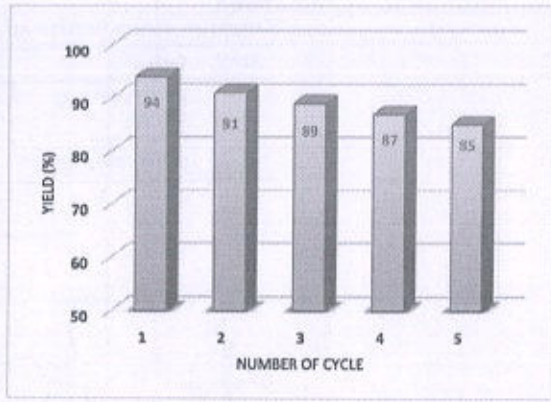



Fig. 7. Reusability study of $[HEPiPYBSA]^+HSO_4^-$ catalyst.

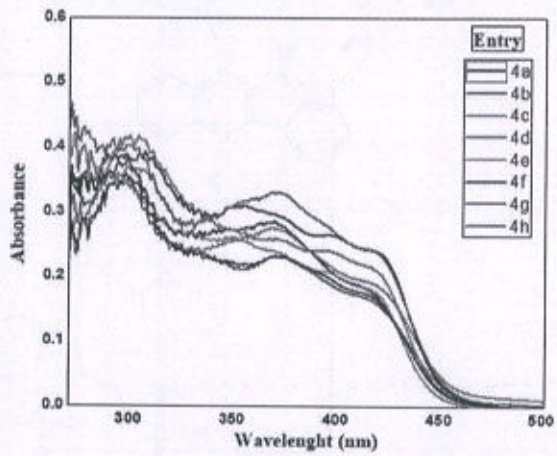



Fig. 8. Solid state UV absorption spectra of compounds 4a to 4h.

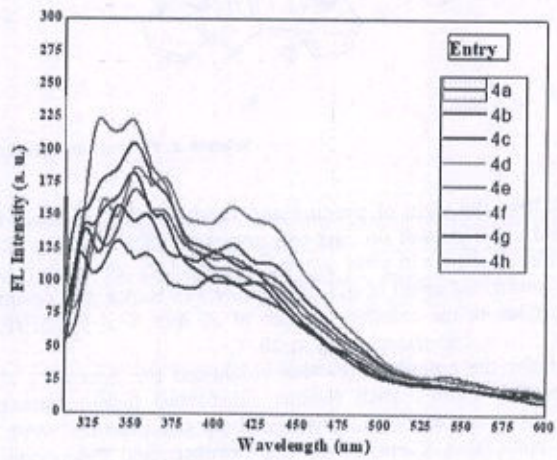



Fig. 9. Solid state fluorescence emission spectra of compounds 4a to 4h

and blue shift is observed due to presence of electron donating group. The extensive conjugation and typical functional groups on synthesized triazoles made them suitable for photoluminescence study. Considering this, all synthesized derivatives were subjected to UV absorption and fluorescence emission characterization in solid state.

The UV absorption study revealed that, all compounds exhibited UV absorption in the range of 300–310 nm [illegible]

$\pi^*$ electronic transition. The fluorescence was then recorded for all synthesized derivatives at 300–310 nm corresponding to UV absorption maxima. Remarkably, all compounds exhibited fluorescence emission in the wavelength range of 325-450 nm with a large shift of 350–375 nm from the excitation wavelength. Conceivably, the 7-methyl-4′-phenylspiro[indeno[1,2-b]quinoxaline-11,3′-[1,2,4]triazolidine]-5′-thione exhibited the highest intensity with the largest shift due to phenyl substitution which induced conjugation resulting in high fluorescence absorption.

## 4. Conclusion

Herein, we explicated synthesis of novel Bronsted acid ionic liquid, $[HEPiPYBSA]^+HSO_4^-$ and explored for synthesis of a series of new spiro 1,2,4-triazolidine-5-thiones. The synthesis of ionic liquid was confirmed by NMR ($^1H$ &$^{13}C$) and IR spectroscopy. In DFT analysis, theoretical study for the synthesized IL was carried out and calculation revealed accurate data of these geometries. The plausible mechanism described role of catalyst in formation of product involving acidic site and hydrogen bonding nature of ionic liquids. The catalytic efficiency of ionic liquid was remarkably found up to five reaction cycles. Mild reaction conditions, short reaction time, high yield of product, operational simplicity, use of nontoxic reagent and catalyst are the characteristics features of the present method. The remarkable feature of synthesized indenoquinoxalino tethered spiro 1,2,4-triazolidine-5-thione derivatives displayed significant photoluminescence properties.

## 5. Experimental

### 5.1. General

Various o-phenylenediamine (Sigma-Aldrich), Ninhydrine (Spectrochem), thiosemicarbazide (alfa aesar), 1,2-ethylhydroxylpiperidine, 1,4 butane sultone and all other reagents and solvents were used as received without any further purification. The melting points were recorded on open capillary method and are not corrected. IR spectra were recorded on Bruker alpha spectrometer with range 4000-400 $cm^{-1}$. NMR spectra were recorded on Bruker AV 400 spectrometer (400 MHz for $^1H$ NMR and 100 MHz for $^{13}C$ NMR) in DMSO-$d_6$ using TMS as an internal standard. $\delta$ values are expressed in ppm. The coupling constants (J) were expressed in Hz. The thermal gravimetric analysis (TGA) was obtained on the TA SDT Q600 in the presence of static air. Fluorescence emission spectrum was examined on FP-8300 Jasco fluorescence spectrophotometer. UV-Visible study was recorded on Specord 210 plus UV/Vis spectrophotometer.

### 5.2. Synthesis of ionic liquid [1-(2-hydroxyethyl)-1-(4-sulfobutyl)piperidin-1-ium]hydrogen sulfate $[HEPiPYBSA]^+HSO_4^-$

A solution of 1, 2-hydroxyethyl piperidine in acetonitrile was stirred for 10 min. Then, 1, 4-butanesultone was added drop wise for 5 min. The mixture was then stirred for 18 h at 80 °C. The generated white precipitate was collected by suction filtration, washed with ethyl acetate and dried in oven at 60 °C for 5 h. The obtained precipitate was reacted with equimolar quantity of sulfuric acid at 60 °C in toluene for 7 h. The reaction mixture was then separated in two phases and the final product, $[HEPiPYBSA]^+HSO_4^-$ was obtained as viscous liquid. The synthesized ionic liquid was washed with ethyl acetate (10 mL x 2) to obtain in pure form.

### 5.3. General procedure for synthesis of spiro 1,2,4-triazolidine-5-thiones

A mixture of indenoquinoxalone (1mmol) and thiosemicarbazide/substituted thiosemicarbazide (1mmol) was taken in flask containing 5 mL water:ethanol (6:4 v/v) mixed system and 20 mol% of $[HEPiPYBSA]^+HSO_4^-$. The reaction mixture was then stirred for 3 h at 70 °C. The progress of reaction was monitored by TLC (ethyl acetate: hexane, 0.3: 0.7). After completion of reaction, resulting precipitate was filtered and washed with ethanol (5 mL x 3) and dried in oven (50 °C). The spiro 1,2,4-triazolidine-5-thiones were subjected to record M.P. and further characterization.

## Author statement

We reported synthesis of novel Bronsted acid ionic liquid $[HEPiPYBSA]^+HSO_4^-$ and explored it for synthesis of a series of new spiro 1,2,4-triazolidine-5-thiones. The catalytic efficiency of ionic liquid was remarkably found up to five reaction cycles. However mild reaction conditions, short reaction time, high yield of product, operational simplicity, use on nontoxic reagent and catalyst are the characteristics features of the present method. Furthermore the synthesized indenoquinoxalino tethered spiro 1,2,4-triazolidine-5-thiones displayed significant fluorescent properties. The data and ideas presented in the manuscript are of our own, novel and are not under consideration for publication elsewhere. All the authors are aware of the submission and agree to its publication.

## Declaration of Competing Interest

None.

## Acknowledgment

Author Vikas B. Shinde also grateful to acknowledge Dattajirao Kadam Arts, Science and Commerce College, Ichalkaranji, Maharashtra (India) for their co-operation and support.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.131528.

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Aayushi International Interdisciplinary Research Journal (AIIRJ)
VOL- IX ISSUE- XII DECEMBER 2022 PEER REVIEW e-JOURNAL IMPACT FACTOR 7.331 ISSN 2349-638x

# Physiology of Resistant Isolates of *Fusarium Udum*, Causal Organism of Wilt of Pigeon Pea

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**Abstract:**

*Effect of various sources of carbon, nitrogen, phosphorus, sulphate, salts, micronutrients, vitamins and amino acids on the growth of Fusarium udum was carried out by incorporating them in Czapek Dox Agar medium. Resistant isolate of Fusarium udum which was determined by taking the sensitivity test of Fusarium udum collected from various localities of Maharashtra and Karnataka were selected for this experiment. Plates without any source served as control.*

***Key words:*** *Amino acids, Czapek Dox Agar medium, carbon, Fusarium udum, micronutrients, nitrogen, phosphorus, sulphate, salts, vitamins.*

## Introduction:

Pigeon pea (*Cajanus cajan* (L.) Huth. a member belonging to family Fabaceae is one of the most essential leguminous food crop cultivated in tropical and subtropical countries like, Madagascar, India, Myanmar, Philippines, Australia. India, Myanmar, Malawi, Tanzania and Kenya are the top 5 producers of this crop. Amongst them India holds a major contribution of 90% of total world production. India engages an area of 3.85 million hectare with an annual production of 2.68 million tonnes (Anonymous, 2010). The plant helps in re-establishing soil productivity by atmospheric nitrogen fixation (Reddy et al., 1993).

Pigeon pea is a commercially important neutraceutical crop as it contains high level of amino acids like methionine, lysine tryptophan, vitamin B and proteins. The content of protein in seeds is almost similar to Soybean (*Glycine max*) ,which ranges from 21-28 % (Phatak et al., 1993). Inspite of this, *Cajanus cajan* is affected by various serious diseases and leads to heavy destruction. Pigeon pea is bombarded by numerous bacteria, viruses, fungi but amongst them just a few of them cause a negative impact on the plant. The wilt caused by *Fusarium udum Butler*, is the most destructive disease (Kannaiyan et al., 1985). Genus Fusarium account to the most significant group of ascomycetous fungi whose members are liable for enormous economic loss due to depletion in yield, quality and quantity of pea (Nelson et al., 1983; Leslie and Summerell, 2006). Many members of Fusarium produce type A and B trichothecene mycotoxins that cause toxicosis in humans and animals (Mali et al., 2015). Several *Fusarium* species cause catastrophic diseases on cereal grains (White, 1980; Parry et al., 1995; Nyvall et al., 1999; Goswami and Kistler, 2004), some are responsible for vascular wilts or root rots on many important vegetable, ornamental and field crops (Kraft et al., 1981; Linderman, 1981) while cankers are produced by others on soft and hardwood trees (Bloomberg, 1981; Dwinell et al., 1981, 2001; Wingfield et al., 2008).

## Material and Method:

Fifteen isolates of infected pigeon pea plants were collected from Kolhapur, Sangli districts of Maharashtra and Dharwad, Vijapura (Bijapur) and, Belgavi (Belgaum) districts of Karnataka. The infected plant materials were brought to the laboratory in clean polythene bags, they were cut into small pieces (0.5-1.0cm length) along the symptomatic region of stem, root and leaves, they were subsequently surface sterilized by sequential dipping in 70% ethanol for 30 sec and in 0.1% HgCl2 for 1 min and were later rinsed in sterilized distilled water, and then cultured on Czapek Dox agar (CDA) amended with 25 mg/l of streptomycin.

Plates were incubated at 25± 2°C for 6 days. The plates were observed for fungal outgrowth through the symptomatic parts of plants. After a period of 5-6 days white cottony fungal mass was observed. On the basis of visual morphological and microscopic characters the fungal isolate was identified as

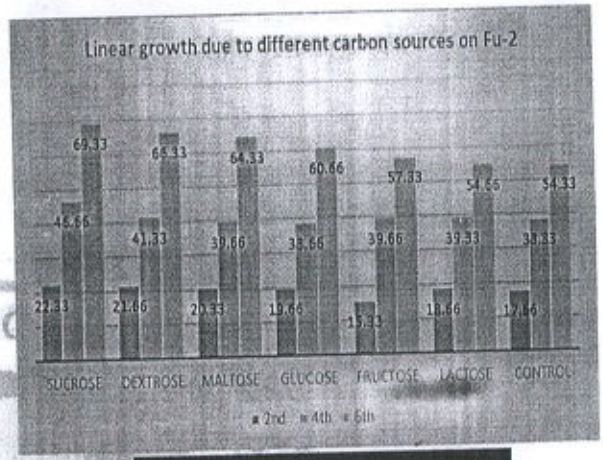
*Fusarium udum* (Butler). *Fusarium udum* was consistently isolated from infected tissues which were purified by single-spore culture method. The sensitivity of *Fusarium udum* was carried out by using Food Poisoning Technique (Dekker and Gielink, 1979) by deploying various concentrations of benomyl a systemic benzimidazole fungicide. The treatment was carried out by preparing benomyl dilutions from 1000 µg/ml stock solution by dissolving it in sterilized distilled water and then mixed in autoclaved Czapek Dox Agar (CDA). The mixture was prepared in proportion of 1:1 and final volume was made up to 30 ml. The media containing Benomyl solution of various concentrations was poured into Petri plates until solidification of media. Pure actively growing fungal mycelium was transferred on the solidified culture media plates by cutting 8 mm diameter discs. These plates were then incubated at 28-30°C in dark and then continuous growth was measured after various time intervals. A plate without benomyl was served as control. For in-vitro experiment, the work was carried out in triplicates. After determining Minimum Inhibitory Concentration (MIC) of benomyl effects of different sources on the development of benomyl resistance was studied in continuous, alternate and mixed pattern along with different fungicide for in vitro experiments.

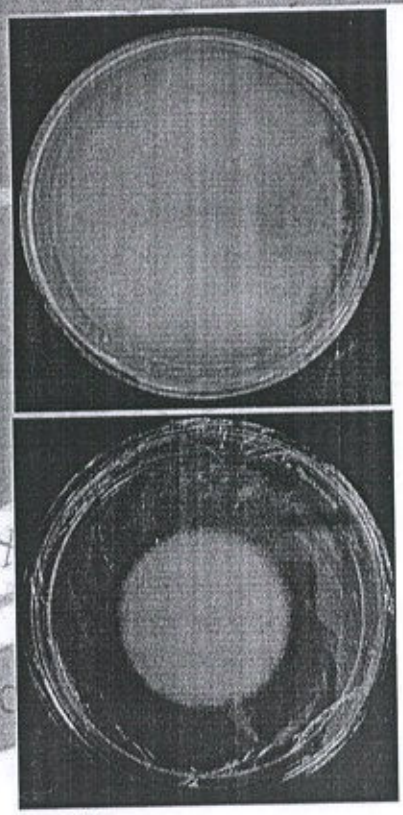
**Result and Discussion:**

**Carbohydrate nutrition**

Different carbohydrate sources like sucrose, fructose, dextrose, maltose, lactose and glucose were amended in Czapek Dox agar at 3% and the linear mycelial growth of the resistant isolate Fu- 2 was recorded. Observations showed that sugars are very much necessary for the growth of both sensitive and resistant isolates. There was maximum increase in the growth of both the isolates over the control. It was found that the resistant isolate's growth rate was higher in comparison with the sensitive isolate. The sensitive and resistant isolate showed a very good rate of growth on sucrose then followed by dextrose, maltose, glucose, fructose and lactose.

**Graph 1. Effect of Different carbon sources on the linear growth (mm) of *Fusarium udum* resistant isolate Fu-2 on Czapek Dox agar.**



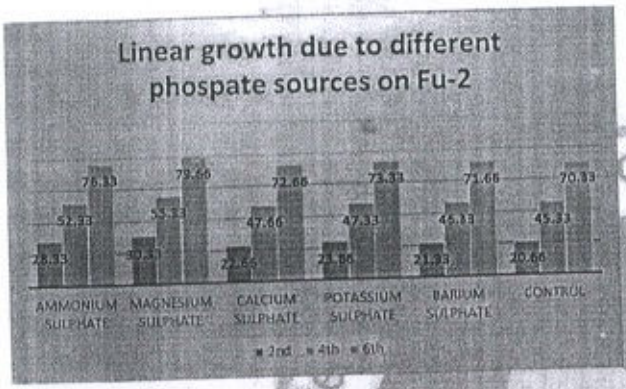
**Nitrogen nutrition**

Various nitrogen sources were utilised to check the effect of nitrogen on the growth of resistant isolate Fu – 2 of *Fusarium udum*. Different nitrogen sources like ammonium, potassium, sodium, magnesium, calcium nitrates and peptone were utilised at 0.2%.

It was observed that there was variation in the growth of both sensitive as well as resistant isolates of various nitrogen sources and in between different incubation periods. The radial mycelial growth of

sulphate helped in good development of *Fusarium udum* followed by ammonium sulphate, calcium sulphate, magnesium sulphate, potassium sulphate and barium sulphate.

**Graph 4. Effect of Different Sulphate sources on the linear growth (mm) of *Fusarium udum* resistant isolate on Czapek Dox agar with benomyl.**



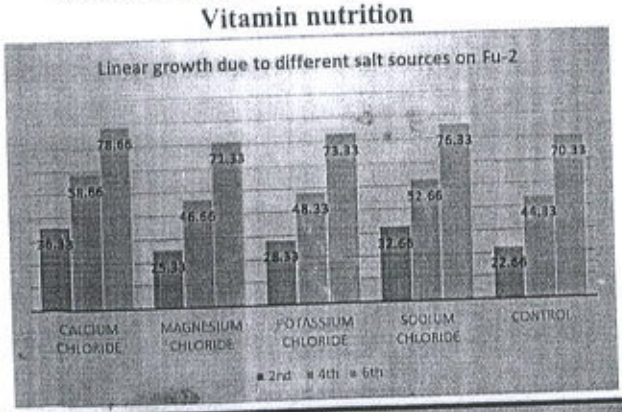

**Effect of salts.**

In total 4 different salts were selected to see the effect on resistant and sensitive isolates of *Fusarium udum*. For the study sodium chloride, calcium chloride, potassium chloride and magnesium chloride were used. They were incorporated at 0.05 mg in Czapek Dox agar medium. Magnesium chloride was found to inhibit the growth of both the isolates.

Growth of resistant isolate Fu- 2 was found to be more luxuriant. It was found that mixture of benomyl along with calcium chloride proved to provide good growth in the resistant and sensitive isolate of *Fusarium udum* followed by sodium chloride, potassium chloride and magnesium chloride.

**Graph 5. Effect of different salts sources on the linear growth (mm) of *Fusarium udum* resistant isolate on Czapek Dox agar with benomyl.**

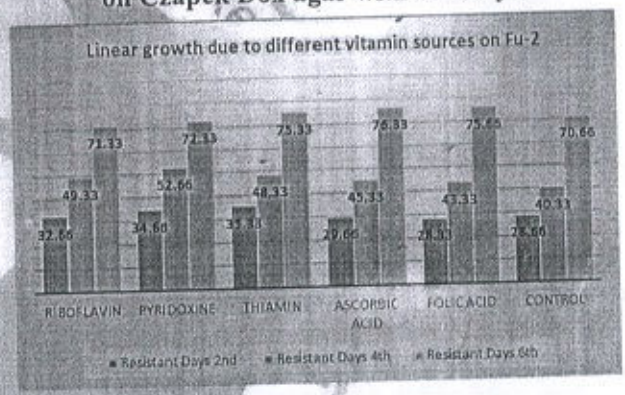
**Vitamin nutrition**




Effect of vitamins was tested on the growth of the resistant isolate Fu- 2. It was mixed in Czapek Dox agar medium at 0.01 mg. It was observed that there was a significant difference on the growth of resistant in the incubation period. Growth of resistant isolate was found to be higher. Plate without any source of vitamin was served as control.

Various vitamins used during the study were riboflavin, ascorbic acid, thiamin, pyridoxine and folic acid. Among all vitamin sources used, ascorbic acid showed a good growth for the resistant isolate.

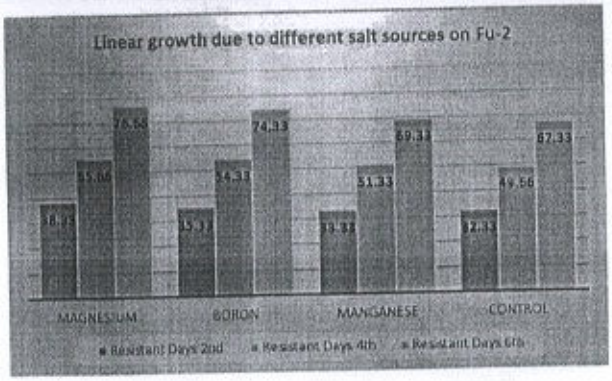
**Graph 6. Effect of different vitamins on the linear growth (mm) of *Fusarium udum* resistant isolate on Czapek Dox agar with benomyl.**




**Effect of Micronutrients**

Effect of different micronutrients was tested on the growth of resistant isolate Fu- 2. It was mixed in Czapek Dox agar medium at 0.01 mg. Magnesium, boron and manganese were used to study the effect when amended with Czapek Dox agar medium. Growth of resistant isolate was found to be higher. Plate without any source of micronutrient was served as control. Magnesium source proved to be good for growth of the isolate. Manganese and boron inhibited the growth of the resistant fungal isolate.

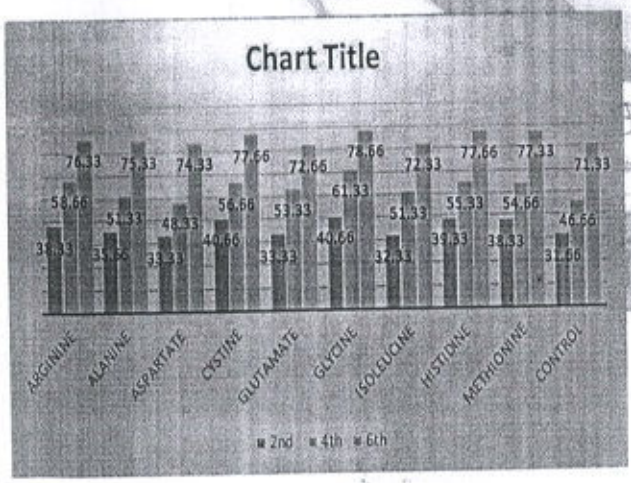
**Graph 7. Effect of different micronutrients on the linear growth (mm) of *Fusarium udum* resistant isolate on Czapek Dox agar with benomyl.**




**Amino acid nutrition**

Various amino acid nutrition were utilised for the study viz. Arginine, Alanine, Aspartate, Cystine, Glutamate, Glycine, Isoleucine, Histidine and Methionine. A significant variation in the growth was observed in resistant isolate Fu -2 . It was mixed in Czapek Dox agar medium at 0.02 mg. Growth of resistant isolate was found to be higher. Plate without any source of amino acid nutrition was served as control. It was interesting to note that almost all the amino acid nutrition showed a good growth on the isolate only isoleucine showed certain amount of inhibition.

**Graph 8. Effect of different amino acids on the linear growth (mm) of *Fusarium udum* resistant isolate on Czapek Dox agar with benomyl.**




**Conclusion:**

Various agrochemicals which are being used by farmers were implied to study their effect to control wilt such as, various fungicides, herbicides, insecticides, antibiotics, micronutrients, salts, fertilizers etc. There are chances that these agro chemicals may influence the development of Benomyl resistance in fungal pathogen hence, both *in vitro* and *in vivo* experiments were conducted.

The foresaid sources show a varying result while treating the resistant isolate of *Fusarium udum* i. e, F-2 in this case. These sources directly or indirectly increase the resistance in the pathogen.

**Acknowledgement:**

The authors are thankful to Dr. Anil Patil, Principal D.K.A.S.C college Ichalkaranji for providing necessary facilities to perform the experiment. The authors are also thankful the the teaching and non - teaching staff of department of Botany D.K.A.S.C Ichalkaranji for moral support.

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# Diversityand Assesment of Indigenous Medicinal Plants From Religeous Sacred Hills in Hatkangaletahsil Dist.Kolhapur, Maharashtra

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**Abstract: -**

*Hatkanangle tahsil is one of the popular and holy places in Kolhapur district. Holy places such as Bahubali hills, Ramling hills, Babu-Jamal darga hills, Dhuleshwar hills, Raspeeth hills (Buddha Hills, Narande hills. are located in outsources of Sahayadri ranges of Western Ghats. The hilly plains in these holy places comprises of deep black soil while slope comprises gravel soil. An attempt has been made to survey and document medicinal plants in religious holy places of Babujamal dararga hills, Ramling hills, Dhuleshwar hills, Bahubali hills and Raspeeth hills of Hatkangale tal., dist. Kolhapur which had great significance in utilization of wild resources pertaining toethno-botanical plants and local medicinal plant (adiabatic). During the survey, 184 plants were assessed by Quadrat method. The plants are found to have medicinal value and remedy for different health problems to local people. It was revealed that, these wild resources (medicinal plants) were utilized by local people for their therapeutic needs. These medicinal plants are very popular among local people and farmers. These plants are utilized frequently in various ailments*

***Keywords:****Babujamal darga hills, Ramling hills, Dhuleshwar hills, Bahubali hills, Raspeeth hills (Buddha hills, Local - medicinal plants, Assessment.*

## Introduction:

It is a fact that over 70-80% of the world population depends on the crude plant drugs to get rid of their health aliments. An Indian material medica includes about 2000 drugs of natural origin derived from different traditional systems and folklore medicines (Narayan et al, 1998) while in modern medicines over 130 drugs originally extracted from higher plants (Dev, 1997). In last few decades, new trends of 'Herbal Drugs' from medicinal plants are becoming more prominently apparent (Dev 1999, Bisset 1994). Now a days it has been estimated that the present global market od indigenous medicine is increasing at the rate of 20% annually (Dev, 1997). The concept of Ayurveda began and flourished between 2500-500 BC in India. The use of medicinal plants were documented in old literature, majority of them were found in Rig-Veda and Athervveda and also in Charaka Sanhita (900 BC), Sushruta Sanhita (600 BC) and Ashtang Hridaya (700 AD). Thus ayurveda is recognized globally by various scientific community.

India is a store house of medicinal plants and there are almost 1250 Indian medicinal plants.(Chattergee and Pakarshi, 1991). Survey of Kolhapur district shows almost 600 plant species of various therapeutic value. Out of them some important medicinal plants are found in the Dhuleshwar hills. Dhuleshwar hillsis one of the holy places of Hatkanangale tahsil. It's situated at 16'45N, 74'22' E and at an altitude of 773 m. from mean sea level. The vegetation is dry deciduous (Yadav and Sardesai, 2000). Duleshwar is the part and parcel of Sahayadri ranges. The plant diversity of Dhuleshwar hills shows different medicinal plants in the form of herbs, shrubs, trees and climbers.

The common medicinal plants screened in this area are as follows

*Gloriosa superb* L., *Discoriea bulbiflera* L., *Plumbago zeylanica* L., *Boerrhavia diffusa* L., *Vitex negundo, Launea procumbens, Lantana camara* L., *Terminalia arjuna, Clerodendrum serratum, Grewia tiliaefolia* etc.

## Material and Method:

The assessment of medicinal plants was studied with the help of quadrat method. The shape of quadrat is usually square. The size of quadrat varies with the type of vegetation to be studied. The quadrat of 10 x 10 m size was laid randomly at three different places and species were recorded with their number in each quadrate. The abundance, density, frequency and frequency percentage of each species were determined by using the standard methods. (Kapur and Rani, 2000). The herbarium specimens were maintained in the laboratory by following standard herbarium techniques. Some selected plants are assed in the following tabulate form.

**Observation:**

**Table.1- Assessment of Ethan medicinal plants by Quadrate analysis:**

| Sr. No. | Name of plants species | Quadrate | | | Total No. of species in all Qua. | Total no. of Qua. studied | No. of Qua. in which species occur | Abundance | Density | Frequency % | Frequency class |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | | | | | | |
| 1. | *Carisa carrandus L.* | 02 | 05 | 03 | 10 | 03 | 03 | 3.33 | 3.33 | 100 | E |
| 2. | *Discoriea bulbifieraL.* | 05 | - | 07 | 12 | 03 | 02 | 4 | 6 | 66 | D |
| 3. | *Plumbago zeylanica L.* | 03 | 02 | 06 | 11 | 03 | 03 | 3.66 | 3.66 | 100 | E |
| 4. | *Commelina benghalensis L.* | 08 | 06 | 10 | 24 | 03 | 03 | 8.0 | 8 | 100 | E |
| 5. | *Lagacea mollis.* | 03 | 15 | 05 | 23 | 03 | 03 | 7.66 | 7.66 | 100 | E |
| 6. | *Acalypha indica L.* | 14 | 15 | 17 | 46 | 03 | 03 | 15.33 | 15.33 | 100 | E |
| 7. | *Lavandula burmanniBenth.* | 11 | 24 | 13 | 48 | 03 | 03 | 16.0 | 16.0 | 100 | E |
| 8. | *Tribulus terrestris L.* | 26 | 37 | 29 | 94 | 03 | 03 | 31.33 | 31.33 | 100 | E |
| 9. | *Stylosathes mucronate Wild.* | 02 | 14 | 17 | 33 | 03 | 03 | 11.0 | 11.0 | 100 | E |
| 10. | *Cyanotis axillaris (L.) D. Don.* | 13 | 07 | 09 | 29 | 03 | 03 | 9.66 | 9.66 | 100 | E |
| 11. | *Spermacoce hispida* | 19 | 27 | 32 | 79 | 03 | 03 | 26.33 | 26.33 | 100 | E |
| 12. | *Rungia Crenata Andrews* | 02 | 26 | 08 | 36 | 03 | 03 | 12 | 12 | 100 | E |
| 13. | *Euphorbia hirta L.* | 21 | 17 | 33 | 73 | 03 | 03 | 24.33 | 24.33 | 100 | E |
| 14. | *Bursera penicillata (Sesse&Moc ex DC.)* | 03 | 05 | 04 | 12 | 03 | 03 | 4 | 4 | 100 | E |
| 15. | *Clerodendrum serratum* | 20 | 23 | 18 | 61 | 03 | 03 | 20.33 | 20.33 | 100 | E |
| 16. | *Panicum americanum L.* | 03 | --- | 07 | 10 | 03 | 02 | 3.33 | 5.66 | 100 | E |
| 17. | *Polygala arvensis Willd.* | 02 | --- | 07 | 09 | 03 | 02 | 3.0 | 3.0 | 100 | E |
| 18. | *Acanthospermum hispidatum L.* | 08 | 11 | 16 | 35 | 03 | 03 | 11.66 | 11.66 | 100 | E |
| 19. | *Gloriosa superba L.* | 06 | 11 | 10 | 27 | 03 | 03 | 9.0 | 9.0 | 100 | E |
| 20. | *Bidens Pilosa L.* | 133 | 106 | 95 | 334 | 03 | 03 | 111.33 | 111.33 | 100 | E |
| 21. | *Evolvulus alsinoides L.* | 06 | 05 | 07 | 18 | 03 | 03 | 6.0 | 6.0 | 100 | E |
| 22. | *Echinops echinatus Roxb.* | 15 | 06 | --- | 21 | 03 | 02 | 7.0 | 10.5 | 66.66 | E |
| 23. | *Opuntia dilleni. Grah.* | 07 | 09 | 08 | 24 | 03 | 03 | 8.0 | 8.0 | 100 | E |
| 24. | *Pergularia arborea. Dennst.* | 03 | --- | 04 | 07 | 03 | 02 | 2.5 | 3.5 | 66.0 | D |
| 25. | *Dodonea viscosa J acp.* | 02 | 04 | 06 | 12 | 03 | 03 | 4.0 | 4.0 | 100 | E |
| 26. | *Iphigenia indica (L.) A Cray* | 02 | 05 | --- | 07 | 03 | 02 | 3.5 | 3.5 | 66.0 | D |
| 27. | *Terminalia arjunaL.* | | --- | 02 | 07 | 03 | 02 | 2.5 | 3.5 | 66.0 | D |
| 28. | *Dichoma tomentosa Causs.* | 03 | 01 | 06 | 10 | 03 | 03 | 9.5 | 9.5 | 100 | E |
| 29. | *Vitex negundo L* | 04 | - | 05 | 03 | - | - | 07 | 02 | 66.0 | D |
| 30. | *Neanotis foetida (Hook.* | 15 | 22 | 18 | 17 | 48 | 25 | 36 | 15 | 100 | E |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | F.) W. H. Lewis. | | | | | | | | | | |
| 31. | Ocimum sanctum.L. | 10 | 10 | 20 | 12 | 56 | 12 | 06 | 36 | 100 | E. |
| 32. | Asparagus racemosus Wild Var. avanica | 05 | 08 | 12 | 03 | 21 | 01 | - | 04 | 66.0 | D. |
| 33. | Withania somnifera L. | 02 | 06 | 03 | 04 | 10 | 08 | 01 | 02 | 66.0 | D |
| 34. | Mimosa pudica L. | 14 | 22 | 20 | 14 | 05 | 36 | 16 | 25 | 100.0 | E |
| 35. | Eclipta alba (L.) Hassk. | 02 | - | 14 | 06 | 08 | 07 | 02 | 03 | 66.0 | D. |
| 36. | Curculigo orchioidesGarten | 10 | 02 | 09 | 03 | 06 | - | 07 | 01 | 66.0 | D |
| 37. | Securinega leucopyrusMuell. | 16 | 12 | 45 | 26 | - | 08 | 06 | 25 | 100 | E |
| 38. | Tinospora cordifolia Miers | 05 | 01 | - | 1 | 03 | - | 01 | 05 | 66.0 | D |
| 39 | Adhatoda zeylanica Medic. | 02 | 04 | 01 | 02 | 03 | 02 | - | 02 | 66.0 | D |
| 40 | Buchnania lanzan Spreng | 20 | 06 | 02 | - | - | 05 | 01 | - | 66.0 | D. |
| 41 | Grewia tiliaefolia. | 02 | - | 02 | - | 04 | 06 | 01 | - | 66.0 | D |
| 42 | Cryptostegia grandiflora R.Br. | 06 | 13 | 04 | 12 | 08 | 09 | 06 | 04 | 66.0 | D |
| 43 | Bacopa monnieri (Micha) | 05 | 02 | 09 | - | 12 | 08 | 22 | 09 | 100 | E |
| 44 | Sterculia urens Roxb. | 10 | 16 | 14 | - | 12 | 20 | - | 15 | 100 | E |
| 45 | Rauwolfia serpentine (Bth) | 02 | 06 | 03 | - | 05 | 04 | 03 | - | 66.0 | D |
| 46 | Boerhavia diffusa(L.) | 06 | 20 | 36 | 14 | 41 | - | 22 | 06 | 100 | E |
| 47 | Aloe vera | 03 | 02 | 06 | 11 | 03 | 03 | 3.66 | 3.66 | 100 | E |
| 48 | Solanum xanthocarpum L. | 02 | 05 | --- | 07 | 03 | 02 | 3.5 | 3.5 | 66.0 | D |
| 49 | Riccinus communis | 05 | 02 | 09 | - | 12 | 08 | 22 | 09 | 100 | E |
| 50 | Euphobia ligularia Roxb. | 20 | 23 | 18 | 61 | 03 | 03 | 20.33 | 20.33 | 100 | E |
| 51 | Sopubia delphiniifolia (l.) G.Don | 08 | 11 | 16 | 35 | 03 | 03 | 11.66 | 11.66 | 100 | E |
| 52 | Rhusmis urensis | 02 | 04 | 01 | 02 | 03 | 02 | - | 02 | 66.0 | D |
| 53 | Abutilon indicum (L.) Sweet | 20 | 23 | 18 | 61 | 03 | 03 | 20.33 | 20.33 | 100 | E |
| 54 | Enicostea axillare L. | | | | | | | | | | |
| 55 | Piper longum L. Sp. | 19 | 27 | 32 | 79 | 03 | 03 | 26.33 | 26.33 | 100 | E |
| 56 | Trichodesma indicum lehn | 13 | 07 | 09 | 29 | 03 | 03 | 9.66 | 9.66 | 100 | E |
| 57 | Mucona pruniens De. | 02 | - | 14 | 06 | 08 | 07 | 02 | 03 | 66.0 | D. |
| 58 | Gymnosporia montanum Benth | 26 | 37 | 29 | 94 | 03 | 03 | 31.33 | 31.33 | 100 | E |
| 59 | Cynotics tuberosa [Roxb] | 15 | 22 | 40 | 41 | 25 | 12 | 40 | 10 | 100 | E |
| 60 | Ruta graveolens L. | 05 | 01 | - | 1 | 03 | - | 01 | 05 | 66.0 | D |
| 61 | Solanum nigrum L... | 02 | 05 | 03 | 10 | 03 | 03 | 3.33 | 3.33 | 100 | E |
| 62 | Cathranthus roseus (l.) G. Don | 03 | 15 | 05 | 23 | 03 | 03 | 7.66 | 7.66 | 100 | E |
| 63 | Gymnema sylvestre R. Br.ex | 02 | 04 | 04 | 10 | 03 | 03 | 3.33 | 3.33 | 100 | E |
| 64 | Launaea pinnatifida Roxb. | 06 | 13 | 04 | 12 | 08 | 09 | 06 | 04 | 66.0 | D |
| 65 | Enicostemma axillare L. | 05 | 01 | - | 1 | 03 | | 01 | 05 | 66.0 | D |
| 66 | Malvarum triuspiatum (R.Br.) A.Gray | 14 | 15 | 17 | 46 | 03 | 03 | 15.33 | 15.33 | 100 | E |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 67 | *Leucasaspera [wild]Link enum* | 14 | 22 | 20 | 14 | 05 | 36 | 16 | 25 | 100.0 | E |
| 68 | *Dodona viscosa (Miller)* | | | | | | | | | | |
| 69 | *Launaea procumbence* (Roxb.) Ramayya & Rajgopal | 03 | 05 | 04 | 12 | 03 | 03 | 4 | 4 | 100 | E |
| 70 | *Desmodium triflorum* (Benth) Drum & Thoth | 02 | 26 | 08 | 36 | 03 | 03 | 12 | 12 | 100 | E |
| 71 | *Indoneesilla echioides L.* | 02 | 05 | --- | 07 | 03 | 02 | 3.5 | 3.5 | 66.0 | D |
| 72 | *Cassia auriculata L.* | 19 | 27 | 32 | 79 | 03 | 03 | 26.33 | 26.33 | 100 | E |
| 73 | *Withania somanifera* L. Dunal | 05 | - | 07 | 12 | 03 | 02 | 4 | 6 | 66 | D |
| 74 | *Lantana camara* auct. non. L. | 15 | 06 | --- | 21 | 03 | 02 | 7.0 | 10.5 | 66.66 | E |
| 75 | *Bouganvillea spectabillusl.* | 05 | 02 | 09 | 1 | 12 | 08 | 22 | 09 | 100 | E |
| 76 | *Polycarpea corymbosa L* | 02 | 04 | 01 | 02 | 03 | 02 | - | 02 | 66.0 | D |
| 77 | *Dodonia viscosa L.* | 05 | 01 | - | 1 | 03 | - | 01 | 05 | 66.0 | D |
| 78 | *Asperags recemosus Wild* | 03 | --- | 07 | 10 | 03 | 02 | 3.33 | 5.66 | 100 | E |
| 79 | *Iphgenia indica L. A. Gray* | 02 | 05 | --- | 07 | 03 | 02 | 3.5 | 3.5 | 66.0 | D |
| 80 | *Malarum triatriatum (R.Br.) A.Gray* | 11 | 24 | 13 | 48 | 03 | 03 | 16.0 | 16.0 | 100 | E |

**Table**

**: -Medicinal uses and Plants listed at Ramling Hills/ Babu-Jamal Hills/ Bahu-bali Hills/Dhulehwar Hills/ Narande Hills/ Raspeeth Hills.**

1. **Ramling Hills Assessment**

| Sr. No. | Name of plants species | Parts used | Medicinal value |
|---|---|---|---|
| 1. | *Carisa carrandus L.* | Fruits, Leaves | Remedy in Hemoglobin loss and anti acidic |
| 2. | *Buchnania lanzan* Spreng. | Seeds, Fruit pulp | Stomach ache |
| 3. | *Vitex negundo* L. | Leaves, Fruits | Poultice of leavesfor inflammation |
| 4. | *Ocimum sanctum* L. | Leaves, Seeds. | Cough and cold |
| 5. | *Cryptostegia grandiflora* R. Br. | Bark, latex and leaves | External application of Poultice, leaves for inflammation, latex against boils, scabies |
| 6. | *Neanotis foetida* (Hook. f.) W. H. Lewis | Leaves | Joint pains, Arthritis |
| 7. | *Launaea procumbence* (Roxb.) Ramayya & Rajgopal | Leaves juice | Heart problems |
| 8. | *Desmodium triflorum* (Benth) Drum & Thoth | ------- | ---------- |
| 9. | *Withania somanifera* L. Dunal | Root, leaves | Tonic, Churn, Nervous disorders medicine. |
| 10. | *Lantana camara* auct. non. L. | Leaves | Injuries |
| 11. | *Gymnosporia montanum* Benth | ---------- | ---------- |
| 12. | *Terminalia arjuna* (Roxb) Wt. & Arn. | Bark, Fruits | Decoction of bark powder, blood purification, decoction with milk for heart problems |

| | | | |
|---|---|---|---|
| 13. | *Dioscoria bulbifera* L. | Tuber | Urinary, energy |

**Babu-jamal Hills.**

| | | | |
|---|---|---|---|
| **14.** | ***Gymnemasylvestre R.Br.ex*** | **Leaves, Roots** | **Diabetic medicine and liver tonic snake bite.** |
| 15. | *Lavandulaburmani /L.bipinnata* | Leaves | Common on hillslopes. |
| 16. | *Burserapenicillata [Sesse] [Moc.ex.D.C.]* | Stem and Wood | Oil is used in medicine. |
| 17 | *Polygala aruensis Wild* | Roots | Peculiar smell of zandu balm. |
| 18 | *Bouganvillea spectabillusl.* | Flower, Leaves. | Used in folk medicine.Anti-ulcerative, Anti- microbial |
| 19 | *Polycarpeacorymbosa L.* | All parts | Occasional on hill slopes on rocky soil |
| 20 | *Malvarumtriuspiatum(R.Br.) A.Gray* | Leaves and seeds | Leaves and seeds are used in Ayurvedic medicines. |
| 21 | *Trichodesma indicum lehn* | Fruits | Common on hill slopes used medicine. |
| 22 | *Leucasaspera [wild]Link enum* | Stem and Roots | Used in many Ayurvedic medicine |
| 23 | *Iphigenia indica L. A.Gray* | Seeds | Common species used as source of Colchicine. |
| 24 | *Tribulusterrestris L.* | Seeds and Leaves | Urinary medicine. |
| 25 | *Enicostemmaaxillare L.* | Leaves & Roots | Joint pain medicine. |
| 26 | *Echinopsechinatus (DC)* | All parts | Skin diseases, cough syrups. |
| 27 | *Dodona viscosa(Miller)* | Laves | Leaves tied along with poultice & muscle pans &swelling. |
| 28 | *Grewiatiliaefoliavahl.* | fruits | Againt intestinal gas problem. |
| 29 | *Cynoticstuberosa [Roxb]* | tubers | Common in moist grassland. |
| 30 | *Cassia auriculata L.* | Leaves and seeds , Roots , Flower | Leaves and seeds are used in Ayurvedic medicines, jaundice and skin diseases. |
| 31 | *Rhusmisurensis* | Leaves and Roots | Used in HIV medicines. |
| 32 | *Bacopamonnierimicha* | All parts | Children cough cold.Historia medicine etc. |
| 33 | *Plumbago zeylanica L.* | All parts | Medicine used in skin diseases |
| 34 | *Malarumtriatriatum(R.Br.)A.Gray* | Leaves and seeds | Leaves and seeds are used in Ayurvedic medicines. |
| 35 | *Withania somniferaL.Dunal* | Root, stem and leaves | Stimulating medicine |
| 36 | *Iphgenia indica L. A.Gray* | Seeds | Common species used as source of Colchicines. |

| | | | |
|---|---|---|---|
| 37 | *Boerhavia difusa L.* | All parts | Swelling and diseases. |
| 38 | *Enicostea axillare L.* | Leaves & Roots | Joint pain medicine. |
| 39 | *Mucona pruniens De.* | Seed | Asthama small insect medicine |
| 40 | *Asperagus recemosus Wild* | Leaves, roots. | Urine disease and acidty. |
| 41 | *Abutilon indicum(L.) Sweet* | Leaves, Stem | Ayurvedic medicine |
| 42 | *Cathranthus roseus (l.)G. Don* | Bark and Seeds | Bark and seed used in Aurvedic medicine specially stomach disorder. |
| 43 | *Dodonia viscosa L.* | Leaves | Leaves tied along with muscle. |
| 44 | *Rutagraveolens L.* | Stem | Oil used in medicine. |
| 45 | *Solanum nigrum L..* | Fruits, seed | Used in medicine. |
| 46 | *Piper longumL.Sp.* | Fruits | Dried, Unripe fruits and roots used in native medicine. |
| 47 | *Launaea pinnatifida Roxb.* | Roots ad leaves | Health tonic |
| 48 | *Sopubia delphiniifolia(l.) G.Don* | Leaves | Common in grassland & Wet field. |
| 43 | *Euphobia ligularia Roxb.* | Latex, Stem. | Used in Ayurvedic medicine. |
| 50 | *Riccinus communis* | seeds | Used in dental medicine, snake bite |
| 51 | *Solanum xanthocarpumL.* | All parts | Used in medicine |
| 52 | *Sterculia urens Roxb.* | Bark, Leaves. | Cough, Diarrheic, bone medicine. |
| 53 | *Commelina benghalensis L.* | Leaves,tubers | Skin disease medicine |
| 54 | *Aloe vera L.* | Leave | Cough, juice anti-inflammatory. |
| 55 | *Lagacea mollis* | Leaves | Skin disorder, Fever |
| 56 | *Acalypha indica L.* | Stem, Leaves, Roots | Anti-bacterial, anti-ulcer |
| 57 | *Stylosathes mucronate Wild.* | Whole plant | Antimicrobial |
| 58 | *Cyanotis axillaris (L.) D. Don* | Whole Plant | Boils and Ascites |
| 59 | *Spermacoce hispida* | Root, Stem, Leaves | Urinary infections, internal heat |
| 60 | *Rungia Crenata Andrews* | Whole Plant | Diuretic, Antimicrobial |
| 61 | *Euphorbia hirta L.* | Leaves, Roots, Stem | Dysentery, Jaundice, Pimples |
| 62 | *Clerodendrum serratum* | Whole Plant | Cough, Cold. |
| 63 | *Panicum americanum L.* | Leaves | Jaundice, Diabetes |
| 64 | *Acanthospermum hispidatum* | Leaves and Flowering Top | Jaundice, malaria, Vomiting |
| 65 | *Gloriosa superb L.* | Tubers and Seed | Antiperiodic and anti-helminthic |
| 66 | *Bidens Pilosa L.* | Leaves | Ulcer, Diabetics |
| 67 | *Evolvulus alsinoides L.* | Whole plant | Blood purifier |
| 68 | *Opuntia dilleni* | Leaves, roots | Diabetes, High Cholesterol |
| 69 | *Pergularia arborea* | Whole plant | Asthma, bronchitis |

| | | | |
|---|---|---|---|
| 70 | *Dodonea viscosa* | Leaves, Roots, Stem | Antimicrobial, anti-inflammatory |
| 71 | *Dichoma tomentosa* | Whole plant | Toothache |
| 72 | *Asparagus racemosus Wild Var. avanica* | Roots, Leaves | Upset stomach, anxiety |
| 73 | *Mimosa pudica* | Roots, leaves | Antibacterial, Antivenom |
| 74 | *Eclipta alba* | Root, leaves | Increase digestive system |
| 75 | *Curculigo orchioides Garten* | Roots, Leaves | Arthretis, Knee Joint |
| 76 | *Securinega leucopyrus Muell.* | Roots, Leaves | Wound healing |
| 77 | *Tinospora cordifolia* | Whole plant | Fever, Bone facture |
| 78 | *Adhatoda zeylanica* | Leaves, roots, Flower and Bark | Cold, cough |
| 79 | *Rauwolfia serpentine (Bth)* | Leaf and root extract | Mental disorder |
| 80 | *Indoneesilla echioides* | | Goiter, Liver disease |

**Conclusion:-**

It is evident from the survey of indigenous medicinal plants, which were assessed from theholy places of Hatkanagle tahsil viz., Ramling hills, Babu-Jamal hills, Bahu-bali hills, Dhuleshwar hills, Narande hills, and Raspeeth hillsthat about 184 plants are found to be having traditional medicinal importance. All of themedicinally important plants were locally used for remedies against different ailments and curing the diseases.

**Acknowledgement:**

Author is thankful to the joint secretary, UGC (WRO) Pune, for providing financial assistance through granting minor research project. Authors is also thankful to Principal, Dr. Milind Hujare. and Laboratory Staff for their technical help in present investigation.

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SCIENTIFIC PUBLISHERS

JETB Journal of Economic and Taxonomic Botany
Volume 46 Issues 3 & 4
September & December, 2022
ISSN: 0250-9768

# WHAT IS THE BOTANICAL IDENTITY OF *SOH-PHLONG*?

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## Introduction

*Flemingia* Roxb. ex W.T.Aiton (Fabaceae) is one of the genera related to the cultivated pigeon pea, *Cajanus cajan* (L.) Huth. The genus occurs in the Old World tropics (Mabberley, 2017) and consists of 44 species and two varieties (Roskov *et al.*, 2013; Gavade *et al.*, 2019). *Flemingia vestita* Benth. ex Baker is an important herbaceous, tuberous, medicinal plant and commonly known as *'Soh-phlong'*. In Khasi language (Meghalaya), *'soh'* means fruit; *'phlong'* means grass, referring to its juicy tubers growing in a soil tufted with grass and weeds (Pandey *et al.*, 2019). There are two forms of this species, i.e. one wild and another cultivated; they differ in habit, leaflets, flowers and pods (Singh & Arora, 1973). The tubers of *Flemingia vestita* are consumed by the local people of northeast India to cure worm infections. The farmers of Meghalaya (Khasi hills) cultivate it as a tuber crop. Tubers are sold in the local market at the rate of Rs. 100–300 per kg (Talang *et al.*, 2019). The tubers are eaten raw by Khasi and Jaintia tribal people as a source of starch (pers. obs.). This species is distributed in Himachal Pradesh, Uttarakhand, Assam and Meghalaya (India) and Nepal (Gavade *et al.*, 2019), China and parts of southeast Asia.

Notwithstanding, Fern (2014), Gawade *et al.* (2019), Nivedhitha *et al.* (2019) and Pandey *et al.* (2019) identified *'Soh-phlong'* to be *Flemingia procumbens* Roxb. So, there exists a contradiction in the published literature and in the field. During the revisionary study of the genus *Flemingia* in India, we also realized that many workers had reported *F. vestita* as *F. procumbens* (Press *et al.*, 2000; Ren & Gilbert, 2010; Fern, 2014; Gawade *et al.*, 2019; Nivedhitha *et al.*, 2019; Pandey *et al.*, 2019), or synonymized the former with *F. procumbens* (Sanjappa, 1992; Roskov *et al.*, 2013). *F. procumbens* Roxb. is an under-shrub without tubers and found in Sal (*Shorea robusta* C.F. Gaertn) forests of Brahmaputra plains and Uttar Pradesh. Therefore, it becomes necessary to establish the botanical identity of the economically important *F. vestita* Benth. ex Baker, and elucidate whether it is identical with, or different from *F. procumbens* Roxb., as conceived by certain taxonomists. For easy identification of these two species, a brief description and a photo plate comparing the species side-by-side are provided along with the diagnostic features in a tabular form (Plate 1; Table 1). Furthermore, Wight's illustration of *F. nilgheriensis* which he was named it as *F. procumbens* created confusion amongst the subsequent workers; this issue is also discussed.

**Table 1.** Distinguishing characters of *Flemingia procumbens* and *F. vestita*

| Characters | *Flemingia procumbens* | *Flemingia vestita* |
|---|---|---|
| Habit | Procumbent shrub | Decumbent herb |
| Roots | Simple, non-tuberous, non-starchy | Tuberous, starchy |
| Leaflets | Equal to or longer than petiole | Shorter than petiole |
| Petioles | 2–3 cm long, winged | 4.5–6.5 cm long, grooved |
| Inflore-scence | Axillary, solitary raceme, 6–12-flowered | Terminal head or capitate, 3–6-flowered |
| Flowers | 0.8–0.9 cm long | 1.5–1.7 cm long |
| Bracts | 0.2–0.3 × 0.12–0.2 cm | 0.6–0.7 × 0.25–0.3 cm |
| Fruits (Pods) | 1–1.2 ×0.45–0.5 cm, exerted the from calyx | 1.2–1.3 × 0.4–0.5 cm, included within calyx |
| Seeds per pod | Two | One |

### *Methodology*

For the present investigation, *F. vestita* was collected from Shillong (Meghalaya) and *F. procumbens* from Kishanpur Wildlife Sanctuary (Uttar Pradesh). The voucher specimens were deposited in the Shivaji

University Herbarium (SUK) at Kolhapur, India. Identity of the species was confirmed after detailed taxonomic study based on the examination of fresh material, type specimens and other herbarium specimens housed at ASSAM, BSD, CAL, DD, K, LY, MH, SUK and WII.

### *Soh-phlong*: Origin and Diversification

Robert Blinkworth, a plant collector for Nathaniel Wallich first collected this legume from Kumaon in 1826 and 1827. Graham proposed the name *Dolichos vestitus* Graham to Blinkworth's plant in Wallich catalogue and did not validate the name (Wallich, 1828). Later, Baker (1876) validated Graham's name and proposed a new combination *Flemingia vestita* in Hooker's *Flora of British India*. While doing so, he reported this species from the Himalaya (Shimla, Kumaon and Garhwal to Khasi hills) and stated that this species was cultivated for its edible roots. On the other, Watt (1890) had never seen it under cultivation; he stated that the species to be growing wild in association with a wild mung (*Vigna vexillata* (L.) A.Rich.) in Shimla. The roots were collected and eaten raw by herd boys while attending to the cattle.

The center of origin and diversification of *Flemingia* is Indo-Burmese region (Mukerjee, 1953). The wild forms of *F. vestita* have been found in Himachal Pradesh, Meghalaya and Uttarakhand, Nepal (Gavade *et al.*, 2019) and China, Laos, Philippines and Vietnam (Sa & Gilbert, 2010). The center of origin of *F. vestita* could be north-eastern region of India since the wild and cultivated forms of this species are found therein. Recently, Mattapha *et al.* (2021) reported this species from Thailand.

### Uses

The tubers of *F. vestita* have an agreeable flavour, sweet and juicy. The skin of tubers is removed by washing frequently in water, and fresh/raw tubers are eaten with salt and chilli powder by the local people of Khasi and Jaintia hills. The tribal people eat raw tubers regularly which help in stomach aches, dysentery and getting rid of intestinal worms. The tubers are rich in carbohydrates, protein, and the elements iron, phosphorus and calcium. They contain more protein as compared with cassava and sweet potato, the important root crops of the tropics (Gangwar & Ramakrishnan, 1989). The skin of the tuber is effective against many tapeworms and it is also used as fish poison (Singh & Arora, 1973). *F. vestita* plays an important role in nitrogen fixation (up to 250 kg/ha per year) and improves soil fertility. Inter cropping with cabbage helps to improve the yield of the latter. Commercial products like biscuits, candies, chocolates, chips, flakes, health drinks '*Sohph-drink*' and soups, jams, jelly, multigrain products, peanut butter, wafers, etc. can also be prepared from tubers of *Soh-phlong* (Pandey *et al.*, 2019). The antihelmintic activity of *F. vestita* tubers was well established (Yadav *et al.*, 1992; Roy & Tandon, 1996; Tandon *et al.*, 1997, 2003; Pal & Tandon, 1998; Kar *et al.*, 2002, 2004).

### Taxonomic treatment

The present study intends to dispel the doubts about the botanical identity of the legume species with the vernacular name '*Soh-phlong*' on one hand and how *Flemingia procumbens* which is at times mismatched with it and which is not '*Soh-phlong*'. Although these two species *F. vestita* and *F. procumbens* appear similar to onlookers, they are unmistakable and easily distinguishable (Table 1; Plates 1 & 2). In fact, these species represent two different subgenera of *Flemingia*, namely ***Rhynchosioides*** Baker and ***Flemingiastrum*** (DC.) Baker, respectively. So, the problem with the botanical identity of '*Soh-phlong*' is wrong application of name and lack of basic understanding of nomenclature. The needed citations of these two species and specimens examined during the present investigation are furnished as evidence. For a more detailed description and specimen citations about *F. vestita*, one can refer to Gavade *et al.* (2019). The following is the key to segregate the two species which are being misidentified.

**Key to *Flemingia procumbens* and *F. vestita*:**

1a. Plants decumbent herbs; tubers present; pod 1-seeded ..... *F. vestita*

1b. Plants procumbent shrubs; tubers absent; pod 2-seeded ..... *F. procumbens*

***Flemingia procumbens*** Roxb., Fl. Ind. (Roxburgh) 3: 338. 1832.

*Maughania procumbens* (Roxb.) Mukerjee, Bull. Bot. Soc. Bengal 6(1): 20. 1953 (as *Moghania procumbens*).

*Lectotype* (designated by Gavade *et al.*, 2016): Flowering specimen in Roxburgh drawing no. 1893 (K) [http://apps.kew.org/floraindica/home.do]. (Plate 1)

Procumbent shrubs, up to 30–45 cm long, branched. *Roots* non-starchy, slender, elongated. *Leaves* digitately trifoliolate, 7.4–8.6 cm long; stipules 2, 0.7–0.8 × 0.1–0.15 cm, lanceolate; petioles 2–3 cm long, winged; leaflets 3, 4.9–5.4 × 2.1–2.4 cm, obovate, acute or acuminate at apex, the central cuneate at base, laterally oblique at base, margin ciliate, hairy on ventral surfaces; dorsally glabrous, densely hairy on nerves, gland-dotted; glands orange-red. *Inflorescence* an axillary, solitary raceme; 6–12-flowered; peduncles 4–5 cm long. *Flowers* 0.8–0.9 cm long, pedicels 0.2–0.22 cm long; bracts 0.2–0.3 × 0.12–0.2 cm, ovate to lanceolate, acuminate, many nerved, gland-dotted, hairy. *Fruit* a pod, 1–1.2 × 0.45–0.5 cm, exserted

from the calyx, beaked, turgid, densely hairy, 2-seeded; beak 0.1 cm long. *Seeds* 2, 0.35 × 0.35 × 0.25 mm, shiny black, rounded; hilum granular, 0.1 cm long, position ± central.

*Flowering and fruiting*: April to May.

*Habitat*: Sal (*Shorea robusta*) forests in shady places, and in grasslands at low elevations of *ca.* 150–200 m AMSL.

*Distribution*: INDIA (Uttar Pradesh and plains of Brahmaputra plains).

*Specimens examined*: INDIA: Assam, Brahmaputra plains, *S. Kurz* s.n. (CAL); Uttar Pradesh, Nepal Frontier distr., Morkatwa, 26.04.1900, *Inayat Khan* 23620 (LY); Bahraich distr., Nandnala, 15.04.1900, *Inayat Khan* 23620a (DD); Gorakhpur distr., 3.03.1898, *Harsukh* 21515a (CAL); 17.04.1898, *Inayat Khan* 21515 (DD, K); Lucknow distr., Chandan Chowki, 23.04.1964, *C.L. Malhotra* 31545 (BSD); Kishanpur Wildlife Sanctuary, 14.05.2017, *S.K. Gavade* 1347 (SUK); Dudhwa National Park, 04.1985, *L.A. Rodgaro* 3875 (WII).

***Flemingia vestita*** Benth. ex Baker, Hook. f., Fl. Brit. India 2: 230. 1876; Gavade *et al.*, Blumea 64: 267. 2019. *Maughania vestita* (Benth. ex Baker) Kuntze, Revis. Gen. Pl. 1: 199. 1891. *Lepidocoma vestita* (Benth. ex Baker) M.R. Almeida, Fl. Maharashtra 2: 105. 1998.

Lectotype INDIA: Kumaon, s.d., *R. Blinkworth* s.n., Wallich Catalogue Number 5545 (K-W001121248); isolectotype CAL (CAL0000067596); G; K (K001081969) and (K001081974). (Lectotype designated by Gavade *et al.* 2019). (Plate 1)

Decumbent herbs, wiry, 45–60 cm long with branched stem. *Roots* tuberous, starchy, globose or cylindrical. *Leaves* digitately trifoliolate, 8–11 cm long; stipules 2, 0.12–0.15 × 0.4–0.6 cm, ovate to lanceolate; petioles 4.5–6.5 cm long, grooved; leaflets 3, 4–4.8 × 3–4.2 cm, obovate to rounded, middle leaflet cuneate at base, lateral leaflets asymmetrical or oblique at base, sparsely hairy on both surfaces, gland-dotted beneath, margin ciliate; glands orange. *Inflorescence* a terminal head (capitate), 3–6-flowered; peduncles 3–6 cm long. *Flowers* 1.5–1.7 cm long; pedicels 0.2–0.3 cm long, hairy; bracts 0.6–0.7 × 0.25–0.3 cm, ovate to lanceolate, acuminate, many-nerved, gland-dotted, hairy. *Fruit* a pod, 1.2–1.3 × 0.4–0.5 cm, included within the calyx, beaked, turgid, glabrous, 1-seeded; beak less than 0.1 mm long. *Seeds* 1, 0.45 × 0.25 × 0.25 mm, black, ellipsoid, hilum less than 1 mm long, position ± central.

*Illustration*: Gavade *et al.*, Blumea 64: 268. f. 13. 2019.

*Flowering and fruiting*: October and December.

*Habitat*: The wild form of this species grows on hill slopes of mountains at elevations of 1600–1800–2100 m AMSL. It is cultivated as a minor crop in Khasi and Jaintia hills of north-eastern region, as well in the north-western region of India.

*Distribution*: India (Assam, Himachal Pradesh, Meghalaya and Uttarakhand) Laos, Nepal, Philippines, southern China and Vietnam.

*Specimens examined*: INDIA: Locality not known: September 1964, *D. Brandis* 3823 (DD). Himachal Pradesh, Kangra distr., *McLeod*, 28.08.2017, *A. Bhatia* 197 (SUK); Shimla distr., Elysian Hill, Simla, 13.08.1877, *Gamble* 4718A (MH); Pulbaha, 19.08.1940, *M.B. Raizada*14261 (DD); Shimla, s.d., *J.R. Drummond* 2518 (DD); Sirmaur distr., 06.08.1986, *R.S. Karki* 82221 (BSD); Solan distr., Banjani, 14.05.1905, *K. Ram* s.n. (DD); On the road Kalka to Dharampur, 15.10.1977, *L.J.G. van der Maesen* 2955 (WAG1972678). Meghalaya, East Khasi Hills distr., s.d., *s.coll. s.n.* (ASSAM); Shillong, s.d., *U. Kanjilal* 7235 (ASSAM); 23.12.2016, *S.K. Gavade s.n.* (SUK). Uttarakhand, Almora distr., Gairar, 17.10.1975, *J.N. Vohra* 57991 (BSD); Champawat distr., Abbott Mount, 23.09.2002, *O. Karki* 98606 (BSD); Dehradun distr. Chakrata, 24.09.1943, *M.B. Raizada* 18294 (DD); Mussoorie, s.d., *J.F. Duthie s.n.* (DD); s.d., *King's collector s.n.* (U1617084); 14.09.1927, *B.L. Gupta s.n.* (DD); 10.1945, *M.B. Raizadas s.n.* (DD); 19.09.1955, *K. Kumari s.n.* (WAG1575083); Pithoragarh distr., Chulkot, 20.07.1951, *F.C. Thomas* 20887 (DD).

**Note on Wight's Icones 987 and 60**

Wight (1846) described *F. procumbens* from Pykara, Nilgiri hills. Then he realized that the name *F. procumbens* was already used by Roxburgh (1832) for an altogether different species. He corrected the error and added the name *F. neilgherrensis* on a slip which was attached to the type specimen at K (Cooke, 1902). However, the name *F. procumbens* remains as it is on Wight's Icon no. 987 published in *'Icones Plantarum Indiae Orientalis'* (Wight, 1846) and Icon no. 60 published in *'Spicilegium Neilgherrense, or, a selection of Neilgherry Plants'* (Wight, 1847) which represent Wight's *F. nilgheriensis*. These two icons are of the same drawing but Wight's icon no. 987 was in black and white whereas icon 60 was in colour. These icons depict similar characters (herbaceous habit, presence of tuber, shape of leaflets and single-seeded pod) with *Flemingia vestita* and hence people were misled to think *F. vestita* as *F. procumbens*. Baker (1876) reported Wight's species as a variety under *F. vestita*, i.e., *F. vestita* var. *nilgheriensis*. Subsequent researchers (Cooke, 1902; Gamble, 1928; Sanjappa, 1992; Gavade *et al.*, 2019) treated this as a distinct species, *F. nilgheriensis* (Baker) Wight ex T.Cooke.

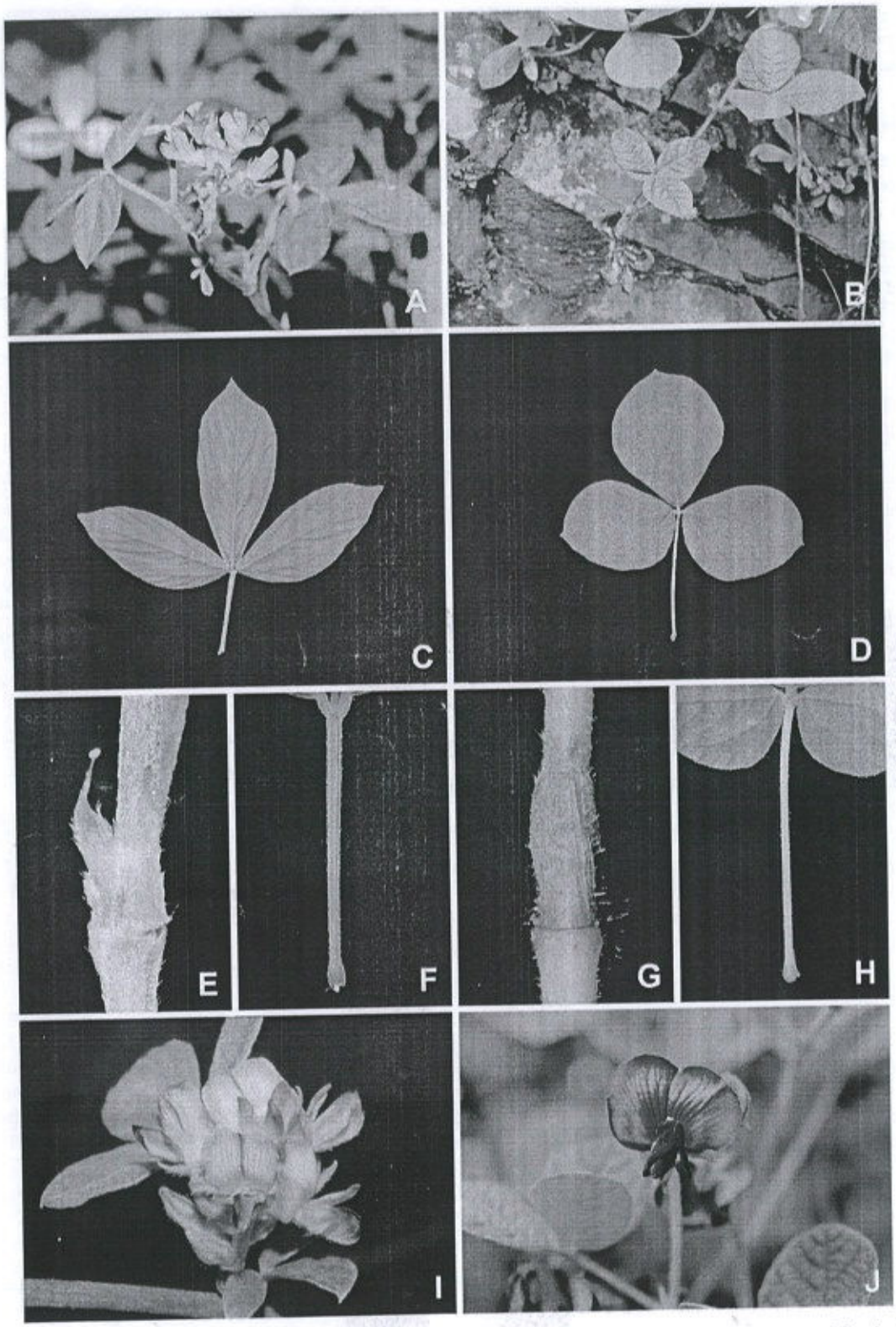


**Plate 1.** ***Flemingia procumbens.*** **A** – Flowering twig; **C** – Leaf; **E** – Stipule; **F** – Petiole; **I** – Inflorescence. ***Flemingia vestita.*** **B** – Flowering twig; **D** – Leaf; **G** – stipule; **H** – Petiole; **J** – Inflorescence.

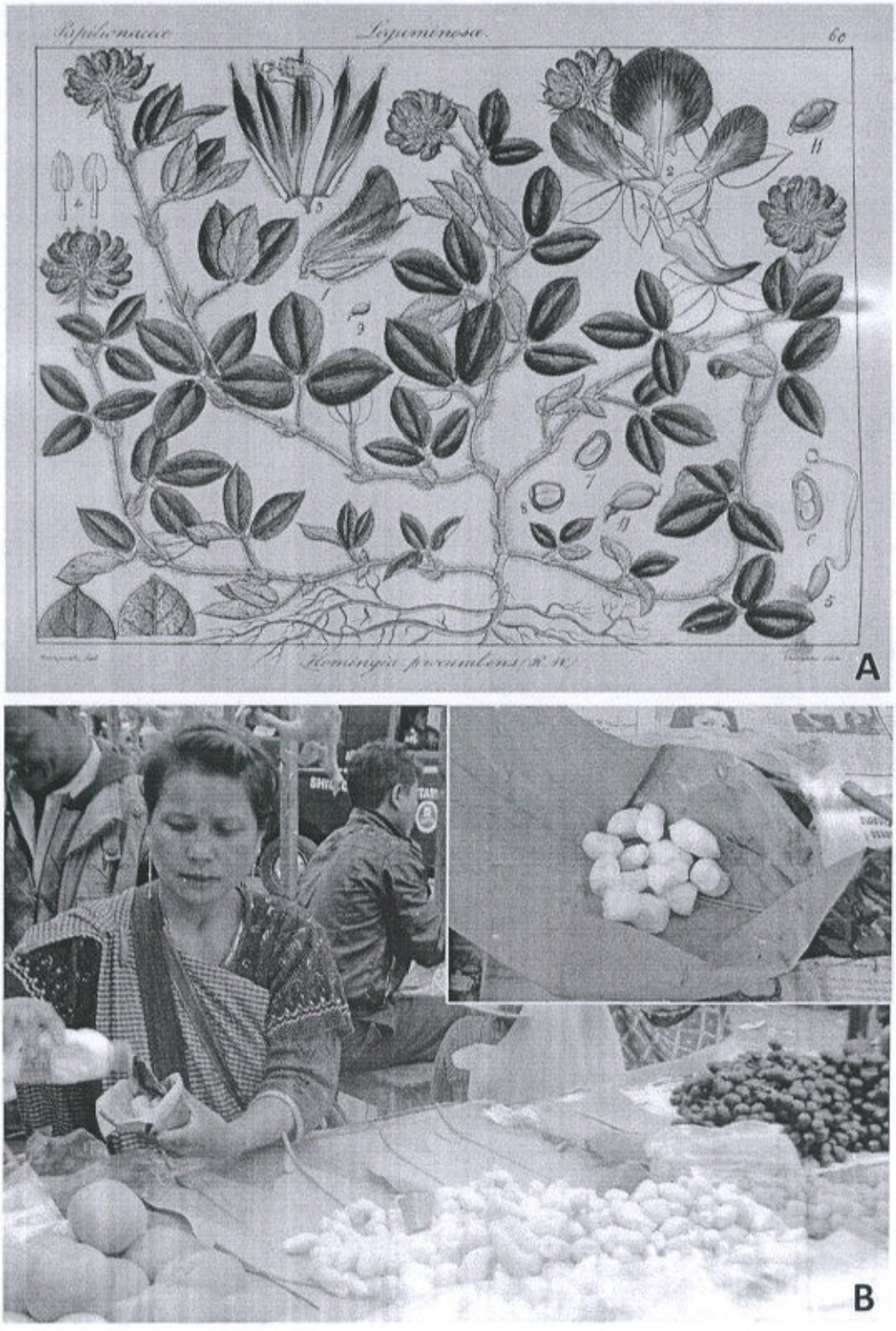


**Plate 2.** ***Flemingia nilgheriensis*** (*F. procumbens* Wight). **A**– Wight's Icon 60 Reproduced with the kind permission of Biodiversity Heritage Library (BHL), Smithsonian Libraries and Archives, Washington, D.C.; **B** – A woman selling *Soh-phlong* (tubers of *Flemingia vestita*) in Shillong, along with exotic fruits.

## Conclusion

*Flemingia vestita* is a member of *Flemingia* subg. *Rhynchosioides*; it shows close affinity with *F. gracilis, F. mukerjeeana, F. nilgheriensis, F. rollae* and *F. tuberosa* (Gavade *et al.*, 2019). *'Soh-phlong'* is a very common tuber crop cultivated in northeast India and also used as a medicinal plant. The wild and cultivated forms of *Flemingia vestita* differ in the size of the plants, leaflets, flowers and fruits. It is a very clearly distinguishable species from *F. procumbens* which is not only found in India but well-distributed in Laos, Nepal, Philippines, southern China and Vietnam. The present work provides the proper and authenticated data for the correct name *F. vestita*. Wight's icon no. 60 represented by Fern (2014) is of *F. nilgheriensis*, not that of either *F. procumbens* or *F. vestita*.

## Acknowledgments

We are grateful to the Head, Department of Botany, Shivaji University, Kolhapur and Principal, Swami Vivekanand Shikshan Santha's, Dattajirao Kadam Arts, Science and Commerce College, Ichalkaranji for providing research facilities. We thank the authorities of ASSAM, BSD, CAL, DD, K, L, LY, MH, SUK and WII for allowing us to consult their herbaria. We also thank Biodiversity Heritage Library (BHL), Smithsonian Libraries and Archives, Washington, D.C. for providing and allow publishing the Wight's icon no. 60. SKG thanks the Science and Engineering Research Board, New Delhi, for financial assistance vide sanction letter SERB/F/201/2014–15 dated 15.05.2014.

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**Received: 15.01.2022 Revised: 08.10.2022 Accepted: 10.11.2022**