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QUANTITATIVE ETHNOBOTANICAL STUDY IN GAFARGAON SUB-DISTRICT AND UNVEILING DRUG CANDIDATES THROUGH MOLECULAR DOCKING AND DYNAMICS SIMULATION APPROACHES

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Abstract

An ethnobotanical investigation was carried out in Gafargaon sub-district (upazila) under Mymensingh district, Bangladesh that unveiled a total of 79 medicinal plant species under 74 genera and 46 families which were used to treat various ailments through 106 formularies. In addition, molecular docking and dynamics simulation studies were performed based on ethnobotanical outcome for the first time in Bangladesh to unveil potential drug candidates. The study revealed that most of the species used for primary healthcare were herbs (44.3%) followed by trees (36.7%), shrubs (10.2%) and climbers (8.8%). Leaves were found to be the most frequently used part (34%) compared to other plant parts. Factor of informant consensus values ranged from 0.975 to 0.984 and the highest value was recorded for respiratory tract disorders (0.984). Maximum number of taxa was unraveled to treat digestive and gastrointestinal disorders. Fidelity level varied from 41.2 to 100%, where 11 species showed 100% fidelity, and the citation frequency was found above 70% for 15 different ailments. Molecular docking study exposed 60% *Stephania japonica* phytocompounds scoring higher than the control drug Ibuprofen (-7.0 kcal/mol) targeting rheumatoid arthritis. The phytocompounds Oxostephanine, Trilobine and Epistephanine were identified as lead drug candidates with binding affinity of -9.7, -8.7 and -8.6 kcal/mol, respectively. Molecular interactions of these compounds were found significant to identify potential drug surface hotspots. Molecular dynamics simulation shed light on regional flexibility profiles and unraveled notable structural stability of the top three phytocompounds. The present study would offer foundational data for identifying potential bioactive compounds that could be utilized in novel drug discovery efforts.

Introduction

Plants with medicinal uses have been a quintessential component of traditional healthcare system since antiquity. Indigenous therapeutic uses of plants have enriched existing traditional medicinal knowledge (TMK) which in turn, developed the pedestal of modern medicines (Pandey and Tripathi, 2017; Mouele *et al.*, 2022). Conservation of this traditional botanical knowledge (TBK) through formal documentation is considered as a key factor to open new avenues for designing and developing novel drugs (Yordi *et al.*, 2022). According to World Health Organization (WHO), ethnomedicine are still the primary sources of healthcare for approximately 80% of the world's population, especially in rural areas of developing countries. Over 50% of all

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pharmaceutical drugs can be traced back to their origins in ethnomedicine (Van Wyk *et al.*, 1997; Suchana *et al.*, 2022). Recently, the use of ethnomedicinal plant-based formularies have gained popularity over the use of synthetic drugs focusing issues, such as safety and efficacy which demands a more rigorous and scientific documentation of indigenous medicinal knowledge (Minnady *et al.*, 2022). Lack of plant identification skill is a hindrance to this endeavor which might results in inappropriate selection of taxa for specific ailment and at this point taxonomic expertise is very much essential (Hadiati *et al.*, 2022). The global market for herbal medicines has prospered as a result of cumulative significance of ethnobotanical research. The worldwide market was valued at approximately US$ 83 billion in 2019, and it is projected to grow significantly, reaching an estimated worth of US$ 550 billion by 2030 (Suchana *et al.*, 2022). This brilliant economic outgrowth is endangered due to some factors including continuous decline in traditional medicinal practices, reduced interests of the younger generation toward traditional treatment systems coupled with rural depopulation, mass deforestation, and migrations of traditional medicinal healers to other jobs (Faruque *et al.*, 2018). All of these facts further necessitate the need of ethnobotanical research to conserve ethnomedicinal values of plants and cultural heritage.

Molecular docking and molecular dynamics simulation (MDS) are *in silico* techniques which facilitate validation of the ethnobotanical findings at the molecular level to shed light on potential lead candidates of specific taxa to carry out drug design works. Molecular docking provides insights into the interactions that occur at the atomic level between a small molecule (referred to as a ligand) and a protein (referred to as a receptor). Molecular dynamics simulation is used to inspect the temporal behavior and movement of atoms and molecules employing equations of motion grounded in classical physics. In Structure Based Drug Design (SBDD), molecular docking and dynamics simulation approaches help to recognize intermolecular interactions, dynamic behavior of complexes, structural alterations, and properties exhibited by molecular systems which drive rational designing of novel inhibitors targeting a wide range of diseases and disorders. These comprehensive *in silico* strategies can save time, cost and labor on the contrary of conventional *in vitro* and *in vivo* investigations (Azim *et al.*, 2020; Ahmed *et al.*, 2022). Very recently, some ethnobotanical studies have attempted these molecular approaches to signify ethnobotanical findings and led a possible strategy for the development of future therapeutics targeting specific ailments based on peoples' perception and practical perspectives (Vijayakumar *et al.*, 2016; Maghfiroh *et al.*, 2021; Abdulrahman *et al.*, 2022).

Janus Kinase 1 (JAK1) is a tyrosine kinase receptor that plays a critical role in the pathogenesis of rheumatoid arthritis when dysregulated via production of pro-inflammatory cytokines which mainly drive disease progression of rheumatoid arthritis (RA). Binding of cytokines facilitates phosphorylation of JAK1 and it gets activated. This activation results in the formation of STAT (Signal Transducers and Activators of Transcription) dimer that is translocated to the nucleus, acts as transcription factor and initiates transcription of genes encoding pro-inflammatory cytokines which ended up with production of malignant protein that causes rheumatoid arthritis. Therefore, inhibition of JAK1 plays a regulatory role in the JAK-STAT pathway to prevent the production of pro-inflammatory cytokines and consequently to stop the disease progression of RA (Tanaka *et al.*, 2022).

In Bangladesh, several efforts have been made to record and document the traditional knowledge of ethnomedicinal plants, leading to a resurgence in folk medicine over the past two decades (Hassan and Khan, 1986, 1996; Mia and Huq, 1988; Alam *et al.*, 1996; Uddin M.Z. *et al.*, 2008, 2015, 2017, 2019; Uddin S.B. *et al*., 2011; Sajib and Uddin, 2015; Hossain and Rahman, 2018). These studies shed light on medicinal plants of particular community, specific diseases or certain areas of Bangladesh. Nevertheless, there are still numerous areas and communities in Bangladesh that have not been explored. Many more medicinal plants used as sources of herbal

drugs by ethnic groups, folk medicinal practitioners (FMPs), and local people are yet to be uncovered from those unexplored areas and communities (Hossain and Rahman, 2018). In the recent past, Rahman *et al.* (2019) conducted a floristic survey on the angiosperm flora of Gafargaon sub-district, however, there has been no ethnobotanical investigation attempted targeting this sub-district. Moreover, molecular docking and dynamics simulation analyses have never been conducted to validate ethnobotanical outcomes in any earlier studies carried out in Bangladesh. Therefore, the current study aimed to employ ethnobotanical protocol to unravel peoples' consensus regarding the ethnomedicinal uses of plants in Gafargaon sub-district via quantitative analyses. The investigation aimed further to unleash the power of molecular docking and dynamics simulation approaches in ethnobotany for the first time in Bangladesh to unveil potential lead phytocompounds targeting particular ailment with specific taxon based on informants' consensus which would shed light on future drug design and discovery.

Materials and methods

Study area:

Gafargaon sub-district under Mymensingh district spans an area of 401.16 sq. km. and is situated between latitudes of 24°15' and 24°33' N, and longitudes of 90°27' and 90°39' E. The sub-district shares its borders with Trishal and Nandail sub-districts to the north, Kapasia and Sreepur sub-districts to the south, Hossainpur and Pakundia sub-districts to the east, and Trishal, Bhaluka, and Sreepur sub-districts to the west. Gafargaon experiences a moderate climate, similar to other parts of the district, as it is located near to the Himalayas and falls within the tropical monsoon zone. The temperature in the area varies from 15.1ºC to 34.4ºC, with monthly average minimum and maximum temperatures of 22.3ºC and 31.8ºC, respectively. The average monthly rainfall is 227 mm. There are significant monthly variations in humidity levels, the maximum ranging from 81% to 97%, while the minimum varying from 47% to 79% (Rahman *et al.*, 2019; BBS, 2022). The sub-district boasts a variety of habitats and ecosystems, including wetlands, cultivated lands, *char* (river islands), homestead areas, scrub jungles, and fallow lands. These diverse habitats support a dense growth of angiosperms, which are crucial for the local economy, environment, and primary healthcare system. Many individuals in the region possess traditional knowledge about plants and their uses, which they rely on for their primary healthcare management.

Plant samples and data collection

Plant samples were collated from the study area through multiple field surveys conducted at various seasons between May 2020 and December 2022. Collected voucher specimens were processed using standard herbarium techniques (Hyland, 1972; Alexiades, 1996) and deposited at the Dhaka University Salar Khan Herbarium (DUSH). All the specimens were critically studied and identified by experts, using standard literatures and online databases (Hooker, 1872-1897; Prain, 1903; Dassanayake and Fosberg, 1980-1991; Ahmed *et al.*, 2008-2009; The Plant List, 2013; POWO, 2022; TROPICOS, 2022). Data on medicinal uses of these plants were collected through semi-structured interviews, group interviews, plant interviews, discussions with key informants, and informal conversations with folk medicinal practitioners locally referred to as *Kabiraz* (Alexiades, 1996). A total of 51 informants, including 11 female and 40 male individuals, with an average age of 55 years were interviewed.

Quantitative analyses

Factor of informant consensus (Fic):

To estimate use diversity targeting particular ailments, Fic values were determined using the following formula (Heinrich *et al.*, 1998):

$$\text{Fic} = \frac{Nur - Ntaxa}{Nur - 1} \quad \text{..... (i)}$$

Here, Nur represents number of use reports in each category, and Ntaxa represents number of species in each category.

Citation frequency (CF %):

CF values help to identify the most commonly used medicinal plants in the study area. CF values were estimated employing the following formula (Friedman *et al.*, 1986):

$$\text{CF} = \frac{n}{N} \times 100 \quad \text{..... (ii)}$$

Here, n refers to number of people interviewed citing species, and N represents total number people interviewed.

Fidelity level (FL %):

FL values are useful to identify the plant species that are most preferred by informants for treating specific ailments. FL values were calculated using the following formula (Friedman *et al.*, 1986):

$$\text{FL} = \frac{Ip}{Iu} \times 100 \quad \text{..... (iii)}$$

Here, Ip denotes to number of informants who indicate use of a species for the same major ailment, and Iu refers to total number of informants who mentioned the same plant for any other use.

Active site prediction of the receptor macromolecule

CASTp 3.0 and SCFBio webservers have been utilized to predict active sites of the receptor JAK1 (Kuman *et al.*, 2012; Tian *et al.*, 2018). For prediction, the PDB file was uploaded to these servers after retrieving from the Protein Data Bank with PDB ID "4K6Z". The CASTp 3.0 server was utilized for single cavity-based prediction, whereas the SCFBio server was employed for making predictions on multiple cavity basis.

Molecular docking and interaction analyses

Based on Fic (Factor informant consensus) value and novelty, *Stephania japonica* was selected for molecular docking analysis targeting rheumatoid arthritis. The receptor protein, JAK1 was retrieved from the Protein Data Bank with PDB ID "4K6Z". This transferase is frequently targeted to search for novel inhibitors targeting rheumatoid arthritis (Singh and Singh, 2020). The protein was prepared using AutoDock MGL tool by deleting water and heteroatoms, adding polar hydrogens and Kollman charges and repairing missing atom residues. Subsequently, the protein was energy minimized by SWISS-PDB viewer following Rahman and Ahmed (2022). Afterwards, 30 bioactive phytochemicals of *Stephania japonica* were retrieved from PubChem database (Semwal *et al.*, 2010). Ibuprofen, as the control drug, was retrieved from DrugBank (Grennan *et al.*, 1979). All the phytocompounds and control were prepared as ligands for molecular docking by applying MMFF94 (Merck Molecular Force Field) force field based on earlier study (Ahmed *et al.*, 2023). Molecular docking was performed using the blind docking approach in PyRx software version 0.8. Docked complexes were visualized using Discovery Studio visualizer for molecular interaction analysis (Ahmed *et al.*, 2023).

Molecular dynamics simulation

The flexibility of the ligand-protein complexes that ranked the highest was assessed using the CABS-flex 2.0 server (http://biocomp.chem.uw.edu.pl/CABSflex2), and the results were

presented using RMSF (Root Mean Square Fluctuation). CABS-flex enables rapid simulation of protein flexibility with minimal system requirements showing a strong correlation between the flexibility simulations obtained from this server and NMR results (Kmiecik *et al.*, 2016; Kuriata *et al.*, 2018). CABS-flex provides high-resolution simulations (10 ns) of protein dynamics in conditions close to their native state, making it a valuable tool for real-time evaluation of protein-ligand stability. The simulation in CABS-flex was conducted using the default parameters, consisting of 50 cycles.

Results and Discussion

Diversity of ethnomedicinal plants:

The present study unveiled traditional medicinal knowledge of 79 species belonging to 74 genera and 46 families which were used for 13 major ailments via 106 formularies. A total of 51 informants took part in the participatory rural appraisal (PRA) from diverse profession and age groups (Table 1). The ethnomedicinally important species alongside families and vouchers, their vernacular names, parts used, mode of administration and ailments treated for each species are documented in Table 2. Asteraceae was found to be the most dominant plant family containing the highest number of species (7.5%), followed by Fabaceae (6.3%), Malvaceae (5.1%) and Rutaceae (5.1%). The most frequently used species were herbs (44.3%), followed by trees (36.7%), shrubs (10.2%) and climbers (8.8%) (Fig. 1A). These findings have been found congruent with several other studies where the dominant ethnomedicinal plants were herbs (Uddin *et al.*, 2019; Suchana *et al.*, 2022). Traditional healers use herbs and trees as the most common sources of medicines (Uniyal *et al.*, 2006), which has been supported further by our study. The maximum number of species were prepared as extract (34.9%), followed by paste (21.7%) and decoction (17.9%) prior to administration (Fig. 1B). The percentage of plant parts administered for treating different ailments is shown in Figure 2.

In a study conducted on the ethnomedicinal plants of Barisal district, extracts were reported as the principal mode of administration which showed congruence with our findings (Hossain and Rahman, 2018). However, Faruque *et al.* (2019) reported paste as the chief mode of preparation in Bilaichari sub-district of Rangamati district, which was found to be inconsistent with our study. This inconsistency might be due to the peoples' perception driven by geographical isolation and community composition of the two areas. About 76% of the species were recorded for internal use and the remaining 24% showed external application. Among the parts used, leaves were found to be the most dominant one (34%), followed by roots (14%), whole plants (13%) and fruits (12%) (Fig. 2).

Table 1. Socio-demographic features of the informants in Gafargaon sub-district.

Variables	Categories	Percentage	Variables	Categories	Percentage
Gender	Male	78.4	Religion	Islam	92.2
	Female	21.6		Others	7.8
Age group	< 30	5.8	Profession	Traditional healer	25.5
	31-50	19.6		Farmer	19.6
	51-70	64.8		Day laborer	13.7
	> 70	9.8		Small shopkeeper	13.7
Education	Illiterate	33.3		Others	27.5
	Primary	43.1			
	Secondary	17.5			
	University	6.1			

A few other studies have also demonstrated that leaves are predominantly used by folk medicinal practitioners for various therapeutic purposes (Hossain and Rahman, 2018; Uddin *et al.*, 2019). The inclination towards using leaves in the preparation of herbal medicines by healers might be attributed to the year-round availability of leaves and their ease of collection, storage, processing, and handling (Faruque *et al.*, 2018).

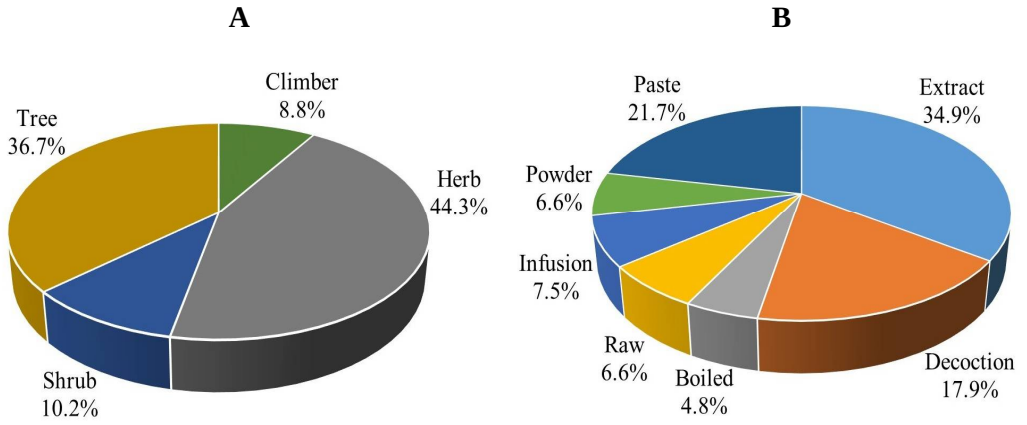

Fig. 1. Comparative analysis of habits and mode of preparation of the recorded ethnomedicinally important plants. A. Classification of species based on habits showing the percentage for treating various ailments; B. Different modes of preparation of the recorded species.

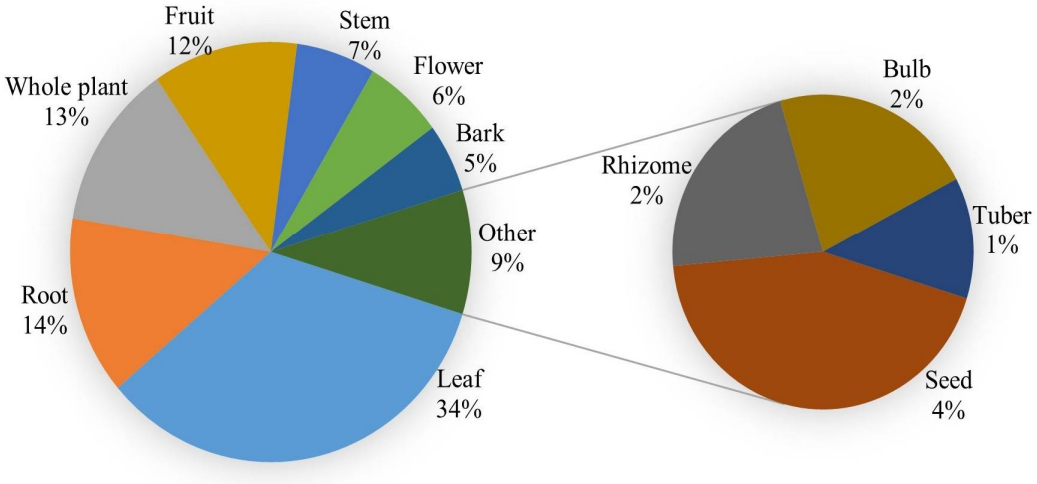

Fig. 2. Use report of different plant parts applied to treat various ailments in Gafargaon sub-district.

Table 2. Ethnobotanical uses of medicinal plants in Gafargaon sub-district with various ailments and mode of administration.

Taxa and voucher	Local name	Parts used	Ailments	Mode of application
Abroma augustum (L.) L.f. (Malvaceae); SSA-229	Ulot Kombol	Stem	Constipation, menstrual problems	Stem aqueous extract is taken after soaking it whole night.
		Root	Dysentery	Decoction of root is mixed with root extract of *Bombax ceiba* and taken orally.
Achyranthes aspera L. (Amaranthaceae); SSA-275	Uuhutlenga, Apang	Whole plant	Infertility problem	2 ml decoction is orally taken thrice a day for three months.
Aegle marmelos (L.) Corrêa (Rutaceae); SSA-270	Bel	Fruit	Diarrhoea	Infusion of fruit pulp is orally consumed.
		Root	Heart palpitation	Decoction of roots is taken internally.
Allium cepa L. (Amaryllidaceae); SSA-244	Peeaz	Bulb	Insect bite	Extract is applied externally to treat swelling and inflammation.
			Oligomenorrhea	Half teaspoon of bulb extract is taken orally with honey early morning on an empty stomach for two weeks.
Allium sativum L. (Amaryllidaceae); SSA-212	Roshun	Bulb	Rheumatoid arthritis	Extract is often taken orally and sometimes boiled for external application.
			Hyperlipidemia	Juice is taken internally.
Amaranthus spinosus L. (Amaranthaceae); SSA-245	Khoirakata	Leaf	Skin inflammation	Paste is applied externally.
		Root	Skin allergy	Decoction is applied externally.
		Whole plant	Jaundice	Decoction is taken orally.
Amorphophallus paeoniifolius (Dennst.) Nicol. (Araceae); SSA-279	Oulkachu	Tuber	Piles	Extract is consumed orally at night.
Ananas comosus (L.) Merr. (Bromeliaceae); SSA-206	Anarosh	Leaf	Helminthiasis	Crushed young leaves are combined with powdered *Areca catechu* roots and mixed with water for oral consumption.
Annona squamosa L. (Annonaceae); SSA-271	Ata	Fruit	Cardiovascular problem	Fruit juice is taken orally along with *Mesosphaerum suaveolens* seeds.
Aphanamixis polystachya (Wall.) R. Parker (Meliaceae); SSA-233	Pitraj	Leaf	Rheumatoid arthritis	Leaf paste is applied externally.
Averrhoa carambola L. (Oxalidaceae); SSA-276	Kamranga	Fruit	Anorexia	Juice is taken orally.
Azadirachta indica A. Juss. (Meliaceae); SSA-219	Nim	Leaf	Allergy	Leaf paste is applied externally, sometimes consumed orally as small tablets.
		Stem	Toothache	Young shoots are used for brushing teeth.
Bombax ceiba L. (Malvaceae); SSA-232	Shimul	Root	Dhat syndrome	Decoction of roots is taken internally, sometimes after mixing with root extract of *Xanthium strumarium.*
Cajanus cajan (L.) Huth (Fabaceae); SSA-208	Aarol, Orohor	Leaf	Jaundice	Juice is taken internally along with coconut water.
			Constipation	Leaf paste is taken internally.
Calotropis procera (Aiton) W.T. Aiton (Apocynaceae); SSA-268	Aahon, Akondo	Leaf	Asthma Body ache	Boiled leaf is inhaled, sometimes taken orally. Leaf juice is taken internally.

Table 2 Contd.

Taxa and voucher	Local name	Parts used	Ailments	Mode of application
Carica papaya L. (Caricaceae); SSA-266	Pabda, pepey	Leaf	Body ache	Leaf juice is taken orally.
			Jaundice	Leaf paste is taken orally.
Cassia fistula L. (Fabaceae); SSA-234	Bandor Lori	Seed	Jaundice	Seed paste is administered internally early in the morning for five consecutive days.
Centella asiatica (L.) Urb. (Apiaceae); SSA-204	Dholmanik, Thankuni	Whole plant	Diarrhoea	Decoction is taken orally.
			Cold and cough	Decoction is internally taken.
Chenopodium album L. (Amaranthaceae); SSA-267	Bottoua Shak	Leaf	Body ache	Leaf paste is orally taken.
		Whole plant	Constipation	Boiled or fried as vegetables.
Chromolaena odorata (L.) King & Rob. (Asteraceae); SSA-277	Boro Heyalmuti	Leaf	Skin cut and laceration	Paste of leaves is applied externally for blood clotting and wound healing.
Cinnamomum tamala (Buch.-Ham.) Nees & Eberm. (Lauraceae); SSA-248	Tej Pata	Leaf	Cold and cough	Boiled leaf extract is taken internally with tea.
Clerodendrum infortunatum L. (Lamiaceae); SSA-253	Bhait	Leaf	Chronic dysentery	Leaf juice is taken orally.
		Flower	Rheumatoid arthritis	Flower paste is taken internally along with ash of coconut shell.
Coccinia grandis (L.) Voigt (Cucurbitaceae); SSA-203	Kauajhingi	Leaf	Dyspepsia and flatulence	Boiled leaf is taken with rice.
		Root	Diabetes	Decoction of roots is taken orally.
Cocos nucifera L. (Arecaceae); SSA-213	Nairol	Fruit	Diarrhoea	Coconut water is taken orally.
Colocasia esculenta (L.) Schott (Araceae); SSA-215	Kachu	Bark	Skin laceration	Applied externally for wound dressing.
Corchorus olitorius L. (Malvaceae); SSA-223	Naillya	Seed	Pox	Seed paste is applied externally along with seed oil of *Sesamum indicum*.
Curcuma longa L. (Zingiberaceae); SSA-209	Oldi, Holud	Rhizome	Diabetes	Decoction is taken internally.
Cuscuta reflexa Roxb. (Convolvulaceae); SSA-262	Swarnolot	Whole plant	Helminthiasis	Extract is orally taken once a day for a few days.
Cyanthillium cinereum (L.) H. Rob. (Asteraceae) SSA-251	Kukshima	Root	Dhat syndrome	Extract is mixed with decoction of *Xanthium strumarium* roots and consumed orally.
Cynodon dactylon (L.) Pers. (Poaceae); SSA-264	Durba	Whole plant	Skin laceration	Crushed parts are mixed with flower extracts of *Nymphaea nouchali* and applied externally.
Datura metel L. (Solanaceae); SSA-257	Dhutura	Fruit	Eczema and skin rash	Raw fruit is eaten in small quantity once a day.
Dendrocnide sinuata (Bl.) Chew (Urticaceae); SSA-220	Chutra	Leaf	Skin irritation and itching	Leaf paste is applied externally.
Dillenia indica L. (Dilleniaceae); SSA-260	Chalta	Fruit	Asthenia	Fruit juice is taken thrice a day.
		Leaf	Dysentery	Extract is taken twice a day for one week.
Diospyros malabarica (Desr.) Kostel. (Ebenaceae); SSA-207	Gab	Bark	Dysentery	Crushed bark is consumed orally with curd twice a day for three days.
Eclipta prostrata (L.) L. (Asteracae); SSA-205	Kalahuta	Whole plant	Skin laceration and wound healing	Applied externally to the affected area, sometimes along with *Cynodon dactylon*.
			Body ache	Extract is taken internally twice a day.

Table 2 Contd.

Taxa and voucher	Local name	Parts used	Ailments	Mode of application
Ficus hispida L.f. (Moraceae); SSA-261	Kudura	Fruit	Diabetes	Raw fruit is eaten, sometimes fruit juice is taken orally.
			Asthenia	Juice is consumed regularly.
Glycosmis pentaphylla (Retz.) DC. (Rutaceae); SSA-250	Motkila	Stem	Toothache	Used to brush teeth, applied externally.
Heliotropium indicum L. (Boraginaceae); SSA-274	Aattir Shur, Hatishur	Leaf	Eczema	Leaf paste is applied externally to the affected part of the body.
Hibiscus rosa-sinensis L. (Malvaceae); SSA-202	Joba	Leaf	Flatulence	Infusion of leaves is taken internally.
		Flower	Dyspepsia	Infusion of flowers is taken internally.
Justicia adhatoda L. (Acanthaceae); SSA-218	Adabasok	Leaf	Cold and cough	Leaf juice is taken orally.
Kalanchoe pinnata (Lam.) Pers. (Crassulaceae); SSA-235	Pathor Shila	Leaf	Burning sensation and body ache	Extract is orally taken.
Lablab purpureus (L.) Sweet (Fabaceae); SSA-255	Shim	Leaf	Dermatitis	Leaf paste is applied externally.
Lawsonia inermis L. (Lythraceae); SSA-239	Mendi	Leaf	Insect bite	Leaf paste is used externally to the affected area.
			Dermatitis	Leaf paste is mixed with banana and calcium hydroxide before external application.
Leucas aspera (Willd.) Link (Lamiaceae); SSA-216	Dol Kolosh, Dondokolosh	Leaf	Rheumatoid arthritis	Boiled leaf is applied externally.
Litsea glutinosa (Lour.) C.B. Rob. (Lauraceae); SSA-256	Kharajora	Leaf	Chronic dysentery	Leaf juice is taken orally.
Mangifera indica L. (Anacardiaceae); SSA-221	Aam	Bark	Jaundice	Decoction is taken orally along with seed and fruit extracts of *Syzygium cumini* and *Ficus racemosa*, respectively.
Mikania cordata (Burm. f.) B.L. Rob. (Asteraceae); SSA-247	Asam Lata	Leaf	Diarrhoea	Extract is taken orally twice a day for a few days.
Mimosa pudica L. (Mimosaceae); SSA-214	Lajonti	Root	Menstrual problems	Decoction of roots is taken internally for twice a day.
Moringa oleifera Lam. (Moringaceae); SSA-236	Sajna	Leaf	Rheumatoid arthritis	Leaf paste is taken, sometimes extract is consumed orally.
Murraya paniculata (L.) Jack (Rutaceae); SSA-258	Kamini	Flower	Body ache	Infusion is taken internally.
Murraya koenigii (L.) Spreng. (Rutaceae); SSA-238	Karipata	Root	Skin inflammation	Root paste is applied externally.
Nigella sativa L. (Ranunculaceae); SSA-243	Kailla jira	Seed	Asthenia	Fried seeds are eaten with rice.
Nymphaea nouchali Burm. f. (Nymphaeaceae); SSA-263	Haluk	Flower	Skin cut and laceration	Floral paste is mixed with plant extract of *Cynodon dactylon* for wound healing and blood clotting.
Ocimum sanctum L. (Lamiaceae); SSA-201	Tulshi	Leaf	Acute cough	Raw leaves are eaten.
		Root	Chronic cough, sore throat	Decoction of roots is taken internally.
Oroxylum indicum (L.) Kurz (Bignoniaceae); SSA-278	Kanaidingi	Fruit	Jaundice	Infusion is taken orally.
		Flower	Jaundice	Infusion is taken orally.

Table 2 Contd.

Taxa and voucher	Local name	Parts used	Ailments	Mode of application
Oxalis articulata Savigny (Oxalidaceae); SSA-240	Khud Manik	Whole plant	Flatulence	Extract is taken orally.
Oxalis corniculata L. (Oxalidaceae); SSA-241	Khud Manik	Whole plant	Flatulence	Extract is taken orally.
Phyllanthus acidus (L.) Skeels (Phyllanthaceae); SSA-225	Orboroi	Leaf	Pox	Leaf paste is applied externally.
Phyllanthus emblica L. (Phyllanthaceae); SSA-265	Aamloki	Fruit	Anorexia	Raw fruits are eaten, sometimes fruit juice is taken orally.
Phyllanthus reticulatus Poir. (Phyllanthaceae); SSA-272	Sitkari	Stem	Helminthiasis	Extract is used internally, sometimes mixed with extract of *Tinospora crispa*.
Piper betle L. (Piperaceae); SSA-227	Pan	Leaf	Constipation	Juice is taken orally, especially by children.
Psidium guajava L. (Myrtaceae); SSA-217	Hobri, Peyara	Leaf	Toothache	Leaf juice is taken internally.
Ricinus communis L. (Euphorbiaceae); SSA-228	Bhenna	Bark	Nausea	Bark is wrapped around the neck of children.
Saccharum officinarum L. (Poaceae); SSA-252	Aakh	Stem	Jaundice	Juice is taken internally, sometimes accompanied with coconut water.
Scoparia dulcis L. (Plantaginaceae); SSA-231	Bondhone	Leaf	Diarrhoea	Leaf juice is taken internally.
		Whole plant	Diabetes	Infusion of whole plant is taken regularly to reduce blood sugar level.
Sesamum indicum L. (Pedaliaceae); SSA-224	Til	Seed	Dysentery	Crushed seeds are taken internally, sometimes seed oil is consumed.
Smilax perfoliata A. DC. (Smilacaceae); SSA-269	Kumarilot	Root	Dhat syndrome	Decoction of root is taken internally.
Stephania japonica (Thunb.) Miers (Menispermaceae); SSA-211	Mochilot	Whole plant	Rheumatoid arthritis	Used to wrap the painful areas of the body, applied externally.
		Leaf	Body ache	Leaf paste is applied externally.
			Diarrhoea	Leaf juice is taken internally.
Streblus asper Lour. (Moraceae); SSA-222	Sheura	Root	Jaundice	Decoction of roots is consumed with the bark extract of *Mangifera indica*.
Swietenia mahagoni (L.) Jacq. (Meliaceae); SSA-226	Mahogoni	Root	Diabetes	Extract is taken internally with plant extract of *Coccinia grandis*.
Tagetes erecta L. (Asteraceae); SSA-242	Genda	Leaf	Toothache	Leaf paste is applied externally.
Tamarindus indica L. (Fabaceae); SSA-254	Tetul	Fruit	Hypertention	Juice is orally consumed after mixing with *Allium sativum* bulb extract.
Terminalia arjuna (Roxb. *ex* DC.) Wight & Arn. (Combretaceae); SSA-230	Arjun	Bark	Cardiovascular problem	Decoction of bark is orally taken with fruit juice of *Phyllanthus emblica* and *Terminalia bellirica*.
Tinospora crispa (L.) Hook. f. & Thom. (Menispermaceae); SSA-249	Padma gurunchi	Leaf	Allergy	Leaf paste applied externally.
		Whole plant	Helminthiasis	Infusion of whole plant is taken orally.
Vachellia nilotica (L.) Hurter & Mabb. (Fabaceae); SSA-273	Babla	Flower	Gastro-intestinal disorder	Extract is taken orally to reduce dyspepsia and flatulence.
Xanthium strumarium L. (Asteraceae); SSA-259	Ghagra	Root	Dhat syndrome	Decoction of root is often mixed with *Bombax ceiba* root extract and taken orally at night.

Table 2 Contd.

Taxa and voucher	Local name	Parts used	Ailments	Mode of application
Xanthosoma sagittifolium (L.) Schott (Araceae); SSA-246	Kailla Kachu	Stem	Skin laceration and wound healing	Extract is applied externally to the affected area.
Zingiber officinale Roscoe (Zingiberaceae); SSA-210	Ada	Rhizome	Digestive disorder	Eaten raw, sometimes infusion is taken orally.
			Cold and cough	Taken orally along with honey and *Nigella sativa* seeds.
			Hypertention	Raw rhizome is taken with tea.
Ziziphus mauritiana Lam. (Rhamnaceae); SSA-237	Boroi	Fruit	Jaundice	Juice is taken regularly.

Quantitative analyses:

Factor of informant consensus (Fic):

A total of 13 major ailments were evaluated using Fic values that ranged from 0.975 to 0.984 (Table 3). For different ailments the number of use reports varied from 102 to 1305, while the number of taxa varied from 3 to 29. Among the various ailments, digestive and gastrointestinal disorders exhibited the highest number of taxa (29), followed by skin diseases (23 taxa), and the lowest number of taxa (3) was recorded for anorexia, colorectal problems, and asthenia (Fig. 3).

Table 3. Consensus of agreement on the uses of medicinal plants among informants.

No.	Category of diseases	Most cited species	No. of use reports	No. of taxa	FIC
1	Respiratory tract disorders (acute and chronic cough, runny nose, sore throat, asthma, bronchitis etc.)	*Justicia adhatoda*	506	9	0.984
2	Cardiovascular diseases (hyperlipidemia, hypertention, arrhythmia)	*Terminalia arjuna*	243	5	0.983
3	Helminthiasis	*Cuscuta reflexa*	179	4	0.983
4	Asthenia (body weakness)	*Dillenia indica*	121	3	0.983
5	Male sexual disorders (dhat syndrome)	*Bombax ceiba*	171	4	0.982
6	Colorectal problems (anal fissure, piles)	*Xanthium strumarium*	108	3	0.981
7	Anorexia (loss of appetite)	*Phyllanthus emblica*	108	3	0.981
8	Female sexual disorders (oligomenorrhea, labor pain, menstrual problems)	*Mimosa pudica*	102	3	0.98
9	Rheumatoid arthritis	*Stephania japonica*	250	6	0.979
10	Digestive and gastrointestinal diseases (gastritis, flatulence, diarrhoea, dysentery, jaundice, constipation, stomach ache)	*Cajanus cajan*	1305	29	0.978
11	Myalgia (general body ache) and toothache	*Chenopodium album*	328	8	0.978
12	Skin problems (inflammation, arthropod sting, prickly heat rash, allergies, eczema, acute and chronic dermatitis, laceration, thermal burning)	*Colocasia esculenta*	979	23	0.977
13	Diabetes	*Curcuma longa*	333	9	0.975

In a recent study in Sherpur sadar and Sreebardi sub-districts, Suchana *et al.* (2022) showed that the highest number of taxa were used in digestive and gastrointestinal disorders which further corroborated our findings. In the present study, the highest Fic value was recorded for respiratory tract disorders incorporating acute and chronic cough, runny nose, sore throat, asthma and bronchitis. *Justicia adhatoda* was found to be the most cited species for this disease category. The second highest value (0.983) was observed in three different disorders, such as cardiovascular diseases, helminthiasis and asthenia. *Bombax ceiba* was found as the most cited species to treat male sexual disorders which was congruent with previous study (Hossain and Rahman, 2018). In the case of rheumatoid arthritis, the Fic value was recorded 0.979 with *Stephania japonica* as the most cited species which was found to be concordant with Mollik *et al.* (2010) who reported the same use in Ashuganj sub-district of Brahmanbaria district.

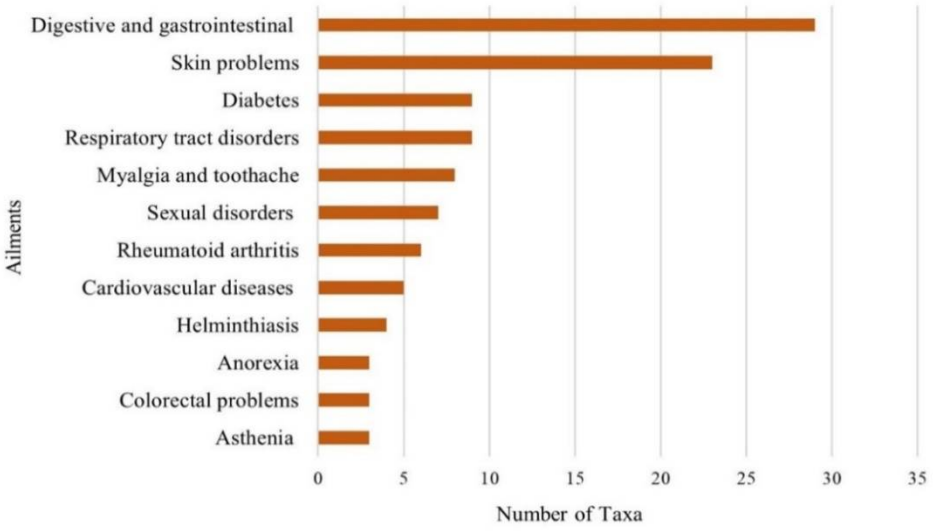

Fig. 3. Number of taxa used to treat major ailments based on informant consensus factor.

Fidelity level (FL):

The present investigation revealed that the fidelity level values ranged from 41.2 to 100% (Table 4). A total of 11 species showed fidelity levels of 100%, *viz*. *Bombax ceiba*, *Centella asiatica*, *Chenopodium album*, *Colocasia esculenta*, *Cuscuta reflexa*, *Cynodon dactylon*, *Lawsonia inermis*, *Phyllanthus emblica*, *Ricinus communis*, *Streblus asper* and *Terminalia arjuna*. The ailments of these top scoring species were anorexia, body ache, cardiovascular disease, dermatitis, dhat syndrome, gastro-intestinal disorder, helminthiasis, jaundice, nausea and skin problems, respectively. Higher level of fidelity for multiple species was found concordant with some recently published studies (Mitu *et al.*, 2022; Suchana *et al.*, 2022).

Citation frequency (CF):

The citation frequency was estimated for all 79 species and amongst them, top 15 scored species were presented in Table 5. Citation frequency was recorded 100% for *Justicia adhatoda*, *Bombax ceiba*, *Cajanus cajan*, *Cuscuta reflexa* and *Stephania japonica*. Citation frequency was found higher than 80% for nine ailments, such as acute and chronic cough, jaundice, skin

laceration, rheumatoid arthritis, helminthiasis, diarrhoea, chronic dysentery, pox and diabetes (Fig. 4). The highest number of taxa was recorded for respiratory tract disorder, while the lowest was found for four ailments including diarrhoea, chronic dysentery, pox and diabetes. Maximum informants cited internal application for the most cited species which was in agreement with previous studies (Hossain and Rahman, 2018; Mitu *et al.*, 2022). Plants with high citation rates could be explored further for the identification of novel phytoconstituents, which could potentially be utilized in the development and discovery of novel therapeutics.

Table 4. Fidelity level values of frequently cited species along with major ailments.

Ailments	Species	No. of informants (I_p)	Total no. of informants (I_u)	Fidelity level (%)
Anorexia	*Phyllanthus emblica*	41	41	100
Body ache	*Chenopodium album*	42	42	100
Cardiovascular disease	*Terminalia arjuna*	48	48	100
Dermatitis	*Lawsonia inermis*	47	47	100
Dhat syndrome	*Bombax ceiba*	51	51	100
Gastro-intestinal disorder	*Centella asiatica*	51	51	100
Helminthiasis	*Cuscuta reflexa*	51	51	100
Jaundice	*Streblus asper*	47	47	100
Nausea	*Ricinus communis*	45	45	100
Blood clotting	*Colocasia esculenta*	51	51	100
Skin laceration and wound healing	*Cynodon dactylon*	51	51	100
Rheumatoid arthritis	*Stephania japonica*	51	75	68.0
Jaundice	*Cajanus cajan*	51	78	65.3
Toothache	*Glycosmis pentaphylla*	36	60	60.0
Gynecological disorder	*Mimosa pudica*	45	80	56.2
Asthenia	*Dillenia indica*	43	81	53.0
Piles	*Xanthium strumarium*	39	75	52.0
Diabetes	*Curcuma longa*	49	96	51.0
Acute and chronic cough	*Justicia adhatoda*	51	102	50.0
Allergy	*Tinospora crispa*	42	102	41.2

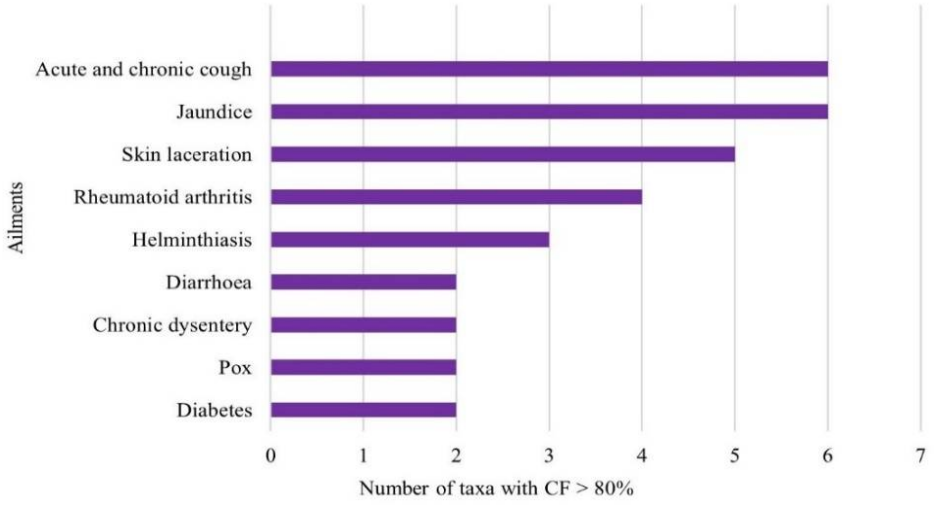

Fig. 4. Number of taxa with citation frequency higher than 80% used to treat various ailments.

Table 5. Citation frequency (CF) of some selected medicinal plant species of the study area.

Species	Ailments	No. of citation	Citation frequency (%)
Justicia adhatoda	Acute and chronic cough	51	100
Bombax ceiba	Dhat syndrome	51	100
Cajanus cajan	Jaundice	51	100
Cuscuta reflexa	Helminthiasis	51	100
Stephania japonica	Rheumatoid arthritis	51	100
Litsea glutinosa	Chronic dysentery	49	96.1
Curcuma longa	Diabetes	49	96.1
Terminalia arjuna	Cardiovascular disease	48	94.1
Lawsonia inermis	Dermatitis	47	92.1
Mimosa pudica	Gynecological disorder	45	88.2
Ricinus communis	Nausea	45	88.2
Phyllanthus emblica	Anorexia	41	80.4
Xanthium strumarium	Piles	39	76.4
Tinospora crispa	Allergy	37	72.5
Glycosmis pentaphylla	Toothache	36	70.5

Active sites of the receptor macromolecule

A single pocket was detected as the active site of the JAK1 protein from the CASTp 3.0 server. The surface area of the active pocket was estimated to be 309.764 $Å^2$ and volume to be 209.690 $Å^3$. The active site resides were Arg27, Leu29, Gly30, Glu31, Gly32, Val37, Ala54, Lys56, Glu73, Leu77, Val86, Met104, Glu105, Phe106, Leu107, Pro108, Ser109, Gly110, Ser111, Glu114, Tyr115, Lys118, Arg155, Asn156, Leu158, Gly168, Asp169 and Phe170. Hence, a total of 28 amino acid residues were predicted as active sites by the CASTp 3.0 server (Fig. 5A).

SCFBio server predicted a total of 39 cavities from where the 8th cavity was considered for current investigation. The volume of the cavity was estimated as 854 $Å^3$. The server did not provide any data on the surface area of the cavity. Cavity point was provided to be -16.514, -2.456 and -5.108 for X, Y and Z axes, respectively. The active site residues were Met139, Asp140, Tyr141, Leu142, Gly143, Ser144, Gln146, Tyr147, Val148, Arg150, Ile176, Glu177, Thr178, Asp179, Lys180, Glu181, Tyr182, Tyr183, Pro198, Glu199, Gln203, Lys205, Phe206, Tyr207, Ile208, Ala209, Val212, Lys278, Glu281, Phe282, Gln283, Pro284, Ser285, Asn286, Thr288, Ser289, Phe290, Gln291, Asn292 and Glu295. Active sites were visualized using Discovery Studio visualizer as shown in Figure 5B.

Molecular docking and interaction analyses:

Molecular docking analysis was performed for bioactive phytochemicals of *Stephania japonica* targeting rheumatoid arthritis. *S. japonica* was selected as it showed a very high level of citation frequency and Fic value. The docking analysis unveiled binding affinities ranging from -5.3 to -9.7 kcal/mol. The control drug Ibuprofen scored -7.0 kcal/mol. Of the 30 phytochemicals investigated, nearly 60% (18) compounds scored higher than the control and the remaining 40% (12) scored lower than the control (Table 6). This indicates the potential of *Stephania japonica* as a source of next generation therapeutics targeting rheumatoid arthritis. The compound Oxostephanine with a molecular weight of 305.3 g/mol showed the highest binding affinity (-9.7 kcal/mol) followed by Trilobine (-8.7 kcal/mol) and Epistephanine (-8.6 kcal/mol). Two dimensional chemical structures of the top three compounds along with the control Ibuprofen are visualized in Figure 6. Among the top three potential drug candidates, molecular weight was

found to be highest for Epistephanine (606.7 g/mol) followed by Trilobine (562.7 g/mol) and Oxostephanine (305.3 g/mol). In contrary, the lowest molecular weight was observed in Viburnitol (164.16 g/mol) among all the phytocompounds, and this compound also exhibited the lowest binding affinity (-5.3 kcal/mol). The docked complexes for the top three compounds along with the control have been shown in Figure 7.

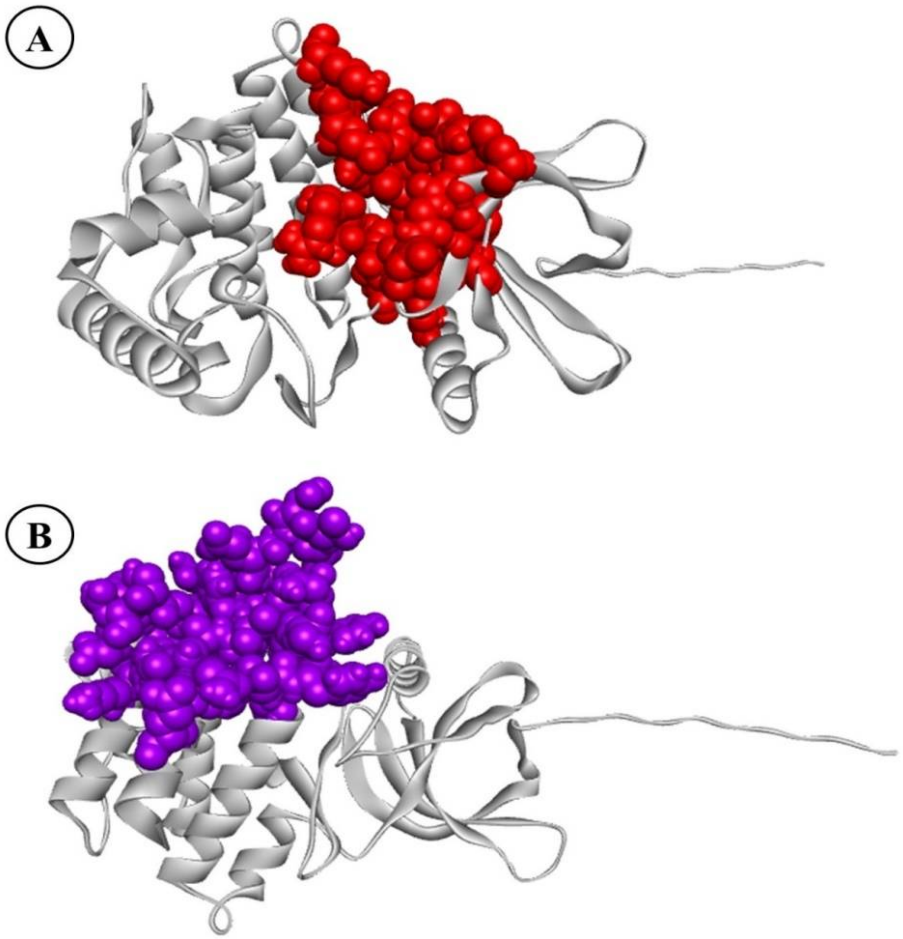

Fig. 5. Active sites of JAK1 receptor macromolecule predicted by CASTp server (A) and SCFBio server (B).

Results of molecular interactions are depicted in Table 7. Molecular interactions were analyzed to justify drug surface hotspots and to find potential active sites in the Janus Kinase 1 receptor which are crucial for drug design and future drug development process (Ahmed *et al.*, 2023). All the three top scoring compounds showed hydrophobic interactions which are vital to ensure stability when these phytocompounds will bind with receptor macromolecule (Rahman and Ahmed, 2022). Both the Oxostephanine and Epistephanine showed conventional hydrogen bonding with the JAK1 protein except Trilobine. Oxostephanine interacted with Leu29 only, while Epistephanine interacted with Asn292 and Ser289 amino acid residues (Fig. 8). These hydrogen bonds play a pivotal role in maintaining stability in ligand-receptor interactions and ensure the specificity of ligand binding (Rahman and Ahmed, 2022).

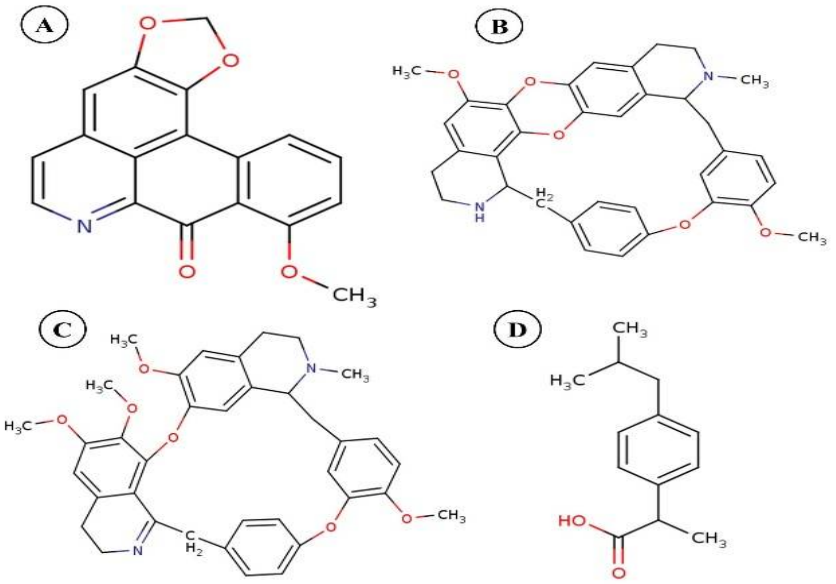

Fig. 6. Two-dimensional chemical structures of three top scoring phytochemicals with the control Ibuprofen. A. Oxostephanine; B. Trilobine; C. Epistephanine; D. Ibuprofen.

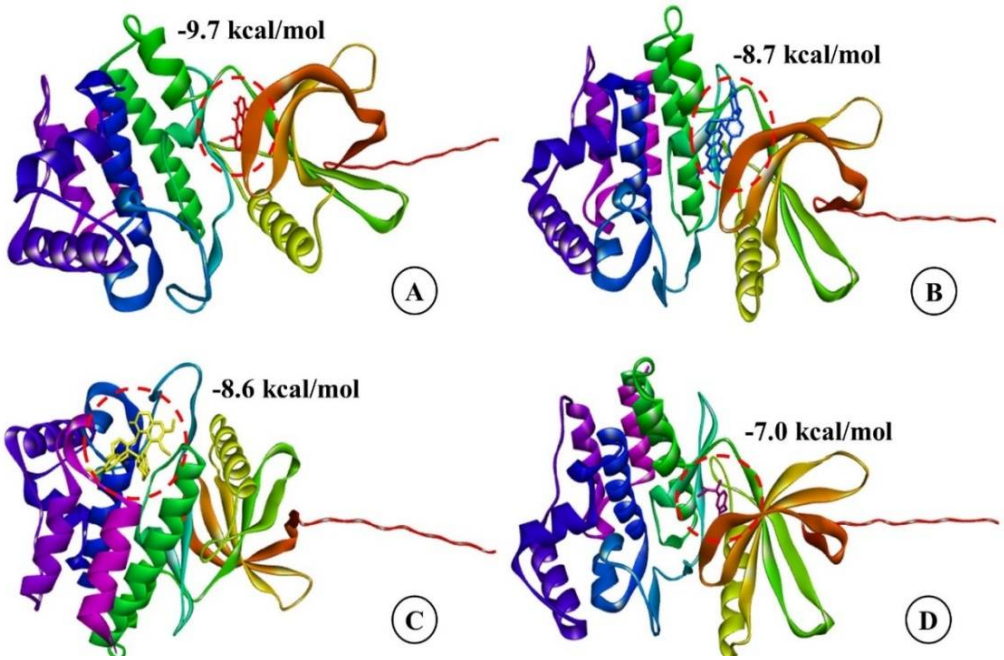

Fig. 7. Three lead phytocompounds of *Stephania japonica* and the control drug with rheumatoid arthritis protein. A. Oxostephanine-complex; B. Trilobine-complex; C. Epistephanine-complex; D. Ibuprofen-complex (control).

Hydrogen bonds donating and accepting regions were further visualized (Fig. 9). Some common residues such as Leu29, Val37, Ala54, Met104 and Leu158 have interacted with Ibuprofen, and two phytochemicals, viz. Oxostephanine and Epistephanine indicating that these amino acid residues of JAK1 could be potential drug surface hotspots for future drug discoveries.

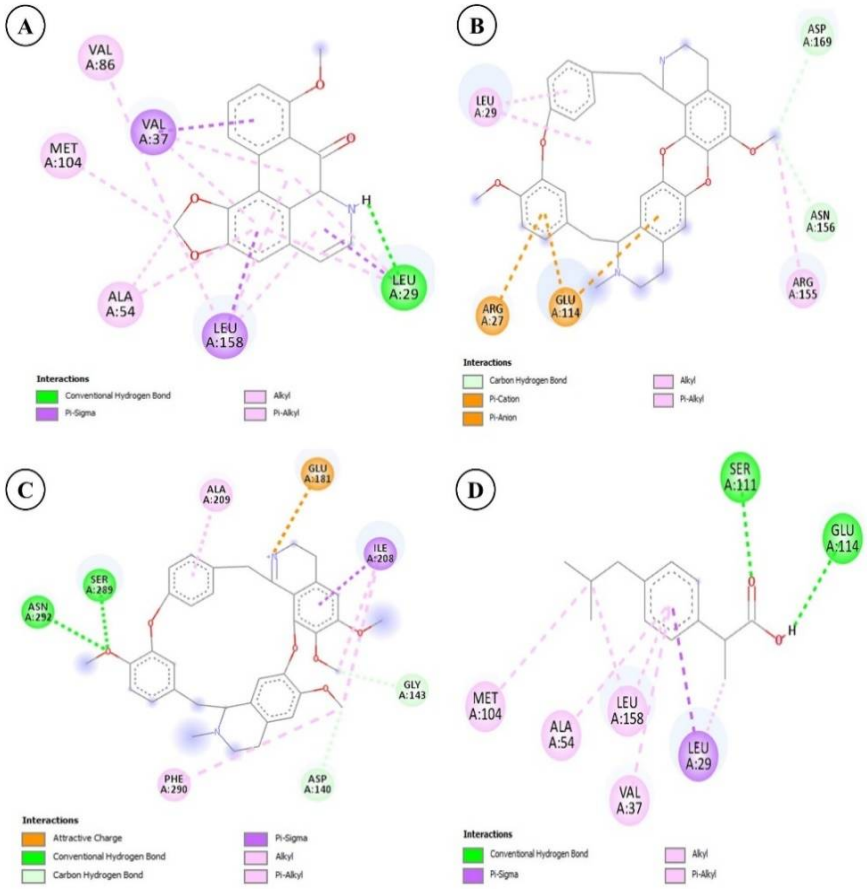

Fig. 8. Two dimensional molecular interactions of the phytochemicals of *Stephania japonica* along with the control. A. Oxostephanine; B. Trilobine; C. Epistephanine; D. Ibuprofen (control).

Molecular docking in conjunction with ethnobotanical research has drawn attention to unveil a new window for drug discovery. In the recent past, molecular docking was applied to a few ethnobotanical studies where Abdulrahman *et al.* (2022) used molecular docking to validate the ethnobotanical outcome targeting Measles in Northern Nigeria. A total of 40 phytocompounds were docked against Measles nucleoprotein from 21 ethnomedicinal plant species and the binding affinities ranged from -1.3 to -9.3 kcal/mol (Abdulrahman *et al.*, 2022). In our study, both the upper and lower thresholds of binding affinity were higher than that of Abdulrahman *et al.* (2022) which justifies accuracy and potentials of *Stephania japonica* phytochemicals targeting rheumatoid arthritis. Vijayakumar *et al.* (2016) conducted an ethnobotanical-molecular docking

survey of traditional Siddha medical practitioners from Thiruvarur district focusing hepatoprotective potentials. Vijayakumar *et al.* (2016) docked three commercial drugs and 12 bioactive phytochemicals from different ethnomedicinal plants against hepatitis B virus receptor, where the binding affinities varied from -5.0 to -8.08 kcal/mol, and Luteolin (-8.08 kcal/mol) was the best scoring compound. Our investigation revealed a significantly higher binding affinity of -9.7 kcal/mol compared to other studies providing additional support to our findings (Abdulrahman *et al.*, 2022; Vijayakumar *et al.*, 2016).

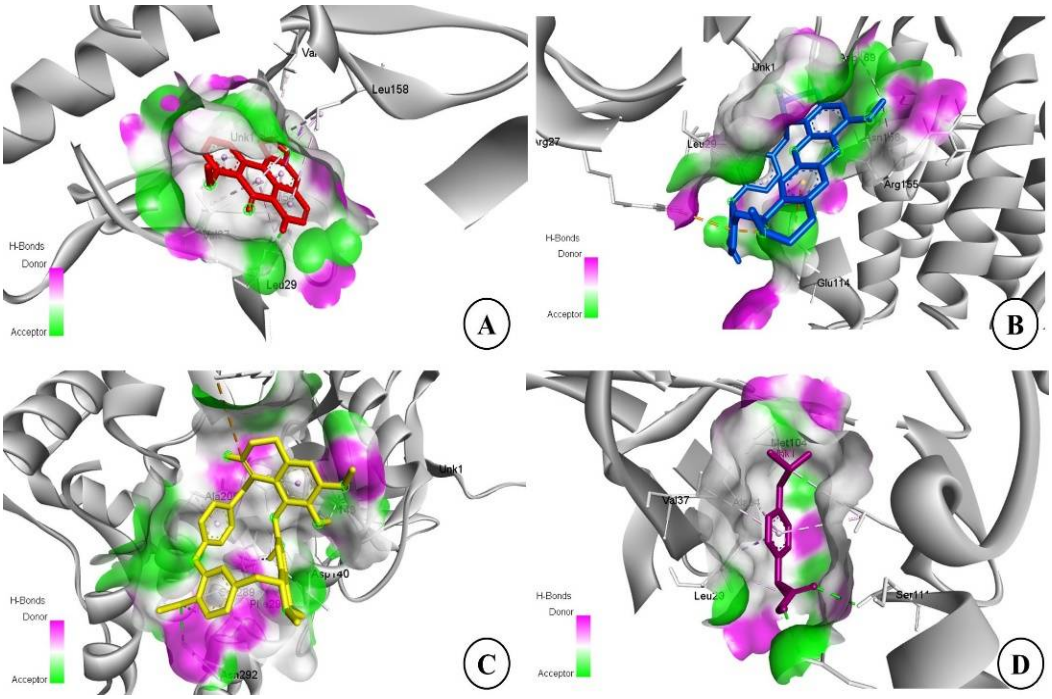

Fig. 9. Three dimensional molecular interactions of the phytochemicals of *Stephania japonica* and the control drug showing hydrogen bonds donating and accepting regions. A. Oxostephanine; B. Trilobine; C. Epistephanine; D. Iburprofen (control).

Molecular dynamics simulation

Structural flexibility analysis unraveled satisfactory results for the tested protein-ligand complexes in comparison with the control drug Ibuprofen-complex. Root Mean Square Fluctuation (RMSF) values were found minimal for all the three lead candidates (Fig. 10). For Oxostephanine, mean RMSF was recorded as 0.96 Å. Trilobine and Epistephanine revealed mean RMSF values of 0.97 Å and 0.90 Å, respectively during the 10 ns simulation trajectory. Ibuprofen showed mean RMSF of 0.82 Å which was slightly lower than the three lead candidates. This close proximity of mean RMSF values indicated nearly same structural stability and flexibility of the tested lead phytocompounds as compared to the control drug. The average RMSF distance in the binding pockets was also estimated that unveiled a mean RMSF of 0.47 Å, 0.65 Å, 0.76 Å and 0.32 Å in Oxostephanine, Epistephanine, Trilobine and Ibuprofen, respectively. All the values were found below the standard threshold of 2.5 Å which denoted very good structural stability of

Table 6. Molecular docking analysis of bioactive phytocompounds of *Stephania japonica* targeting rheumatoid arthritis.

	Phytocompounds	PubChem CID	Molecular formula	Molecular weight (g/mol)	Binding affinity (kcal/mol)
1.	Oxostephanine	343547	$C_{18}H_{11}NO_4$	305.3	-9.7
2.	Trilobine	169007	$C_{35}H_{34}N_2O_5$	562.7	-8.7
3.	Epistephanine	5317122	$C_{37}H_{38}N_2O_6$	606.7	-8.6
4.	Isotrilobine	12310578	$C_{36}H_{36}N_2O_5$	576.7	-8.4
5.	Isochondrodendrine	197726	$C_{36}H_{38}N_2O_6$	594.7	-8.3
6.	Stepinonine	135778935	$C_{36}H_{34}N_2O_7$	606.7	-8.2
7.	Cyclanoline	3082134	$C_{20}H_{24}NO_4^+$	342.4	-8.2
8.	Obamegine	441064	$C_{36}H_{38}N_2O_6$	594.7	-8.2
9.	Bebeerine	12300019	$C_{36}H_{38}N_2O_6$	594.7	-8.1
10.	Tetrandrine	73078	$C_{38}H_{42}N_2O_6$	622.7	-8.1
11.	Fangchinoline	73481	$C_{37}H_{40}N_2O_6$	608.7	-8.1
12.	Oxostephabenine	181354	$C_{27}H_{27}NO_8$	493.5	-8.0
13.	Hypoepistephanine	282017	$C_{36}H_{36}N_2O_6$	592.7	-8.0
14.	Insularine	10348927	$C_{38}H_{40}N_2O_6$	620.7	-7.7
15.	Stebisimine	3083913	$C_{36}H_{34}N_2O_6$	590.7	-7.6
16.	Steponine	15432819	$C_{20}H_{24}NO_4^+$	342.4	-7.5
17.	Cycleanine	121313	$C_{38}H_{42}N_2O_6$	622.7	-7.4
18.	Lanuginosine	97622	$C_{18}H_{11}NO_4$	305.3	-7.3
19.	Aknadinine	159966	$C_{20}H_{25}NO_5$	359.4	-6.8
20.	Stepharine	98455	$C_{18}H_{19}NO_3$	297.3	-6.6
21.	Metaphanine	12312776	$C_{19}H_{23}NO_5$	345.4	-6.5
22.	Homostephanoline	627343	$C_{20}H_{25}NO_5$	359.4	-6.5
23.	Aknadicine	442156	$C_{19}H_{23}NO_5$	345.4	-6.4
24.	Oxostephasunoline	621065	$C_{20}H_{25}NO_7$	391.4	-6.3
25.	Stephasunoline	618654	$C_{20}H_{27}NO_6$	377.4	-6.2
26.	Prometaphanine	91895299	$C_{20}H_{25}NO_5$	359.4	-6.2
27.	Oxostephamiersine	101673501	$C_{21}H_{25}NO_7$	403.4	-6.1
28.	Hasubanonine	442246	$C_{21}H_{27}NO_5$	373.4	-6.0
29.	Epistephamiersine	91895297	$C_{21}H_{27}NO_6$	389.4	-5.9
30.	Viburnitol	101715	$C_6H_{12}O_5$	164.16	-5.3
Ibuprofen (Control)		3672	$C_{13}H_{18}O_2$	206.2	-7.0

Table 7. Molecular interaction analysis of the top scoring three phytocompounds of *Stephania japonica* along with the control drug.

Ligands	Residues in hydrogen bonding	Residues in hydrophobic interactions	Binding affinity (kcal/mol)
Oxostephanine	Leu29	Leu29, Val37, Ala54, Val86, Met104, Leu158	-9.7
Trilobine	No residues	Leu29, Arg27, Glu114, Arg155, Asn156, Asp169	-8.7
Epistephanine	Asn292, Ser289	Asp140, Gly143, Glu181, Ile208, Ala209, Phe290	-8.6
Ibuprofen (Control)	Ser111, Leu158	Leu29, Val37, Ala54, Met104	-7.0

the lead compounds in the binding cavity of the receptor macromolecule. This further justified their potentials to be effective drug candidates against rheumatoid arthritis. Superimposed simulation structures of each complex have been demonstrated in Figure 10.

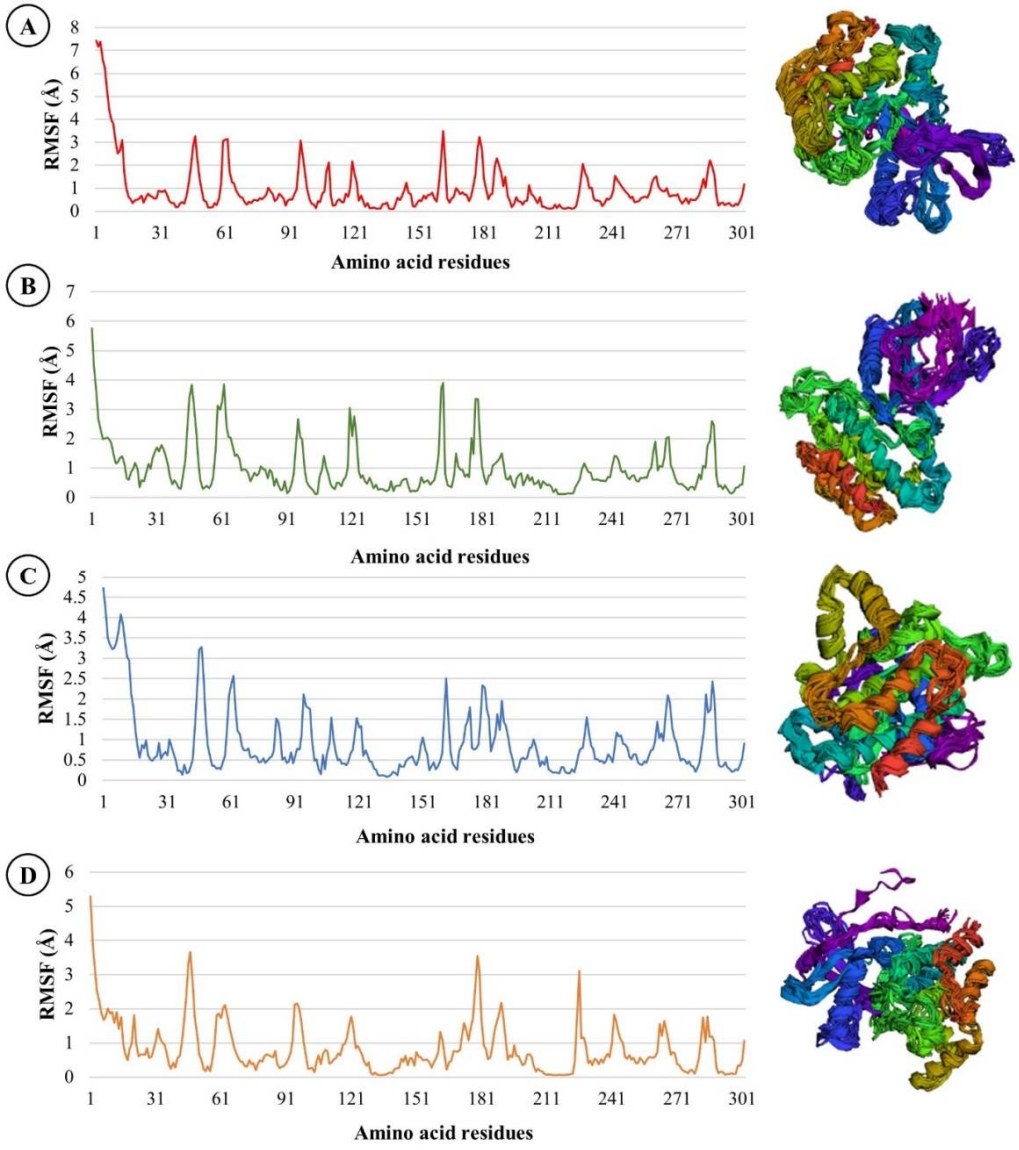

Fig. 10. Molecular dynamics simulation showing superimposed simulated structures and regional flexibility profiles of *Stephania japonica* phytochemicals – Oxostephanine (A), Trilobine (B), Epistephanine (C) and control drug Ibuprofen (D).

This present study combines the principles of molecular docking and dynamics simulation with ethnobotanical knowledge in Bangladesh, marking the first of its kind in this field of research. The study uncovered some novel findings about the traditional medicinal uses of various

plants of Gafargaon sub-district. Remarkably, *Aphanamixis polystachya* leaf paste was found to be effective in treating arthritis; *Calotropis procera* boiled leaf inhalation was used to alleviate asthma symptoms; *Datura metel* raw fruit was traditionally employed to address eczema, and *Xanthium strumarium* root was used to tackle dhat syndrome. The study unveiled several threats to medicinal plant species including habitat destruction and fragmentation, deforestation, over-exploitation, insufficient awareness among local communities about the need for conserving species diversity, and the planting of exotic species. Our findings further highlight the pressing need for conservation efforts and sustainable management practices to safeguard the future of these valuable medicinal plants. To ensure the preservation of valuable medicinal plant species in the surveyed area, various protective measures should be implemented. These might include establishing nurseries to propagate important and endangered medicinal plants, creating distribution maps with precise coordinates for key species, and employing *ex-situ* conservation strategies to safeguard the medicinal plants in the study area, thereby promoting their sustainable use and development.

Species that exhibited the highest citation frequency, fidelity level and Fic value could be subjected to *in vitro* studies for phytochemical screening. In addition, molecular docking and dynamics simulation analyses might open up new avenues for the designing and discovery of novel drugs from *Stephania japonica* phytocompounds to treat rheumatoid arthritis. Our findings provide the baseline data to bridge the gap between traditional healers and scientific communities. Based on our findings, we recommend conducting additional *in vitro*, *in vivo*, and *in silico* studies on the ethnomedicinal plants identified in this venture, in order to further explore their potential for enhancing healthcare management and drug discovery.

Acknowledgements

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