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**CLUSTAL-W PROGRAM FOR NEIGHBORHOOD EVOLUTIONARY  
ANALYSIS OF ANTICANCER MEDICINAL HERBS**

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**Abstract**

Correct identification of medicinally essential plant species is priceless for effective utilization for food and treatment. The aim of the work is to infer the phylogenetic relationships of three medicinal crops sequence and to find out the crucial macro and micro morphological characters for diagnostic purposes. The sequences (*Myristica fragrans*, *Garcinia gummi-gutta*, and *Gymnema Sylvestre*) of the three taxa of our species group have been extracted from the national center for Biotechnology, know-how dwelling web page. The evolutionary historical past was inferred with the aid of utilizing the neighbour joining approach. The morpho-anatomical characteristics are considered as valuable diagnostic factors for authentication of *Gymnema Sylvestre*. It can be concluded that genus *Gymnema* is a plantae group which are intently related as treated previously in present taxonomic programs.

**Keywords:** Clustal-w Program, phylogenetic, Neighbourhood, Evolutionary, Analysis, Anticancer, Medicinal Herbs,

**1. Introduction**

Nutmeg (*Myristica* sp) is native to Indonesia. The genus *Myristica* by many experts is considered as native plants of Indonesia. Nutmeg plants originated from the Banda Islands, Maluku. Nutmeg has ecological and etnomedical significances and is included in older group within angiospermae division, which comprise of newly evolved species [1]. Characterization based on morphological markers of species in nutmeg family is tough, while their similarity of leaves is considered. So identification depends tremendously on small flower's characterization (1 to 4 millimetre in size), which are only obtained from a sufficiently matured plant [2].

*Garcinia* consist of 250 species or more of dioeciously small shrubs to trees of medium size which are a common inherent of low land tropical forests [3]. This genus shows several characteristics which seems to be interesting to biologists. In areas particularly Southeast Asia and Madagascar consist of centers of diversity where this genus significantly shows sympatric species diversity abundantly both in dioecious and agamosperous species which

are enormous in the genus [4]. From an economic point of view, *Garcinia* is most probably known for highly prized mangosteen fruit. (*G. mangostana* L native tree of south-eastern Asia); Also, mangosteen and other species (e.g., *G. gummi-gutta* [L.] N. Robson or “*G. cambogia*”) become the focus in large natural supplement industry and pharmacological studies are formed around these species [5]. *Garcinia* shows extremely diverse floral forms, especially in androecium, found in angiosperms.

*Gymnema* plant genus seems to be important in therapeutic applications and due to its anti-diabetic property; it is often called to be sugar killer [6,7]. Traditional Indian medicine made use of most of these species for disease curing such as stomach ailments, diabetes, constipation, liver disease and water retention [8,9]. *Gymnema* consist of more than 40 species in which due to similar morphological features sharing, some of them are uncategorized. Among them, *Gymnema G. elegans*, *G. montanum*, and *G. sylvestre* have similar morphological characteristics but varying medicinal values. In traditional medicine *G. sylvestre* can replace *G. elegans* for snake bite and diabetes’s treatment [10]. It also has antimicrobial properties. *G. montanum* is also traditionally used for treatment of disorders such as high cholesterol, diabetes, wounds, gastrointestinal ailments, and inflammation. shared Many medicinal values are shown by *Gymnema* species like some journal reports stating involvement of *Gymnema* species’s extracts (*G. inodorum*, *G. sylvestre* , and *G. yunnanense*) in regeneration or repair mechanism of  $\beta$ -cells of pancreas [11,12]. However, the efficiency is dependent on the correct material use. Medicinal plant’s misidentification can lead to serious health issues and is reported in many countries. For example, 63 people were found with symptoms of nausea, vomiting and general malaise prior to herbal tea consumption which was mixed with neurotoxic Japanese star anise consumption, in 2002(*Illiciumanisatum*) [13,14].

This article describes about the medicinal plants, *Mystriga fragrans*, *Garcinia gummi-gutta*, *Gymnema Sylvestre* species which are multiple aligned in Clustalw omega and construct Dendogram and analysis of Phylogenetic evolutionary relationship among these different species.

## 2. METHODOLOGY

### 2.1 Sequence retrieved

The medicinal plant amino acid (protein sequence, *Mystriga fragrance*, *Garcinia gummi-gutta*, *Gymnema Sylvestre* species) sequences are retrieved from NCBI (National Centre for Biotechnology Information).

### 2.2 Clustalw omega (multiple sequence alignment)

It is a new multiple sequence alignment program that make use of HMM profile-profile and seeded guide trees methods for generating alignments between three or more sequences. Substitute pair is used in sequence alignment tools for two sequence’s alignment.

### 2.3 Simple phylogeny

Mostly the first steps are when users set the software input (Amino acid sequences, NCBI database). In following steps, the user can possibly change the default instrument variables. The tool submission step is consistently the end step where the user can identify a heading in relation with an e-mail address for electronic mail notification and outcome. By the submit button utilization the specified information within the form will be simply submitted for launching the tool on the server.

### 2.4 INPUT ALIGNMENT

#### 2.5 Input Window

Phylogeny utilizing an alignment can directly enter into the input field in a supported format. Alignment formats supported incorporate Clustal, FASTA and MSF. Partially formatted or unaligned sequences will not be recognized. To the end of sequence, a return can be added which may aid the simple Phylogeny tool for reorganization of input. Unpredictable results in form of control/ hidden characters can be obtained as yield directly by accessing of word processor's data.

#### 2.6 File upload

Phylogeny uses an alignment uploaded as a file. Alignment file formats supported comprise Clustal, FASTA and MSF. Partly formatted or unaligned sequences are not included usually. A return to the last portion of the sequence can be added, which can help the simple Phylogeny tool for understanding the input better. Form of control/ hidden characters can be obtained as yield directly by making use of word processor's data. Unpredictable results in form of control/ hidden characters can be obtained as yield directly by accessing of word processor's data.

### 2.7 SET PARAMETERS

#### 2.8 Tree format

This determines the outputs that the Simple Phylogeny tool produces

Table 1: Output of the Simple Phylogeny tool

FORMAT	DESCRIPTION	VALUE
DEFAULT	Newick/PHYLIP format tree file	phylip
CLUSTAL	Clustal format file in addition to the PHYLIP tree	nj
DISTANCE MATRIX	Distance matrix file in addition to the PHYLIP tree	dist

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FORMAT	DESCRIPTION	VALUE
NEXUS	NEXUS format file in addition to the PHYLIP tree	nexus

### 2.9 Distance correction

This controls the simple Phylogeny's attempt in correcting the substitutions multiple times on same site. That is made to be 'on' for extra divergent sequences and it has the effect of branch lengths stretching. Reliable corrections of distances are disabled for more divergent sequences.

### 2.10 Exclude gaps

With this option, the places of the sequences within input having a gap is usually be excluded, enabled columns, alignments are forced to take-up the best positions where all sequences' information's are integrated .

### 2.11 Clustering system

This makes use of the neighbour-joining algorithm to assemble trees from the distance matrix. It makes use of the rapid UPGMA tree construction algorithm.

### 2.12 SUBMISSION

#### 2.13 Job title

By giving a name it's viable to find the tool result. The title will be in relation with the results and will show up in almost all of graphical representations of result.

#### 2.14 Email Notification

Running a tool is mostly a combined approach; when the results are available it's immediately delivered to the browser. On dependence to the tool and its input parameters, time will vary accordingly. It is viable to get a notification while completing the job through electronic mail by ticking the area "Be notified through e mail". An electronic mail linked to results may be mailed to the email address noted in the corresponding text box. Email addresses should be valid for E-mail notifications.

Graphical representation of Phylogenetic tree constructed from 20 sequences (Various Medicinal Plants) with the hypothetical root of straight line denoted 100% sequence similarity and branch length denoted non-sequence similarity. Dendogram is generated using Neighbour-joining method. This results show evolutionary relationship between the medicinal plant species.

### 3. Result

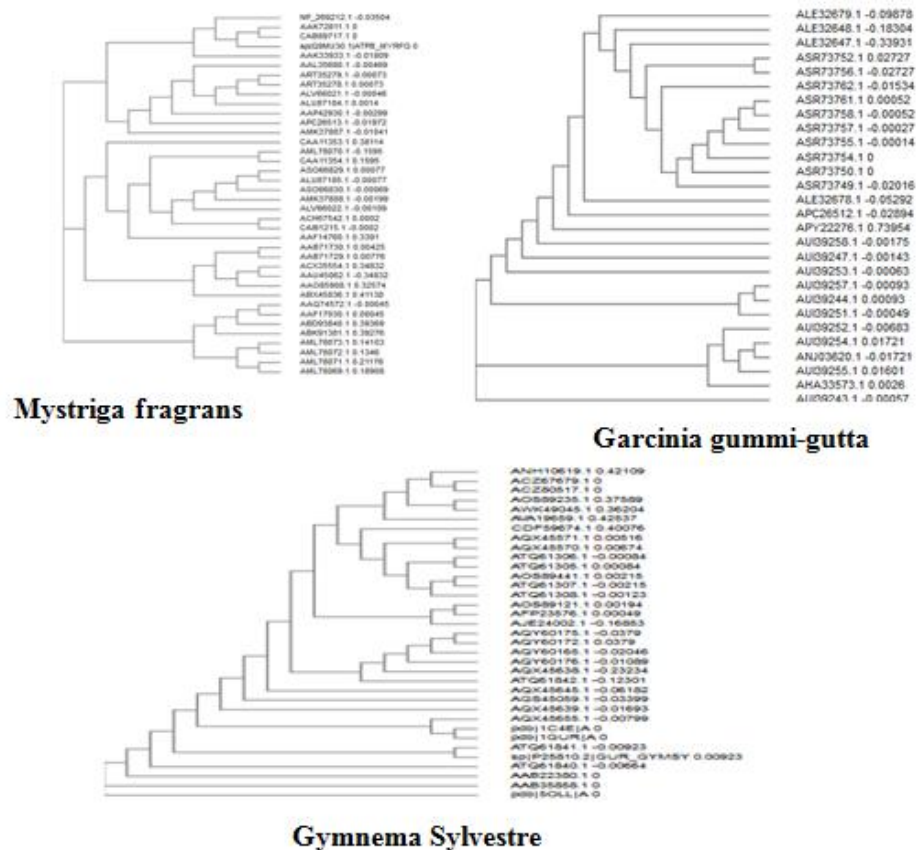


Figure 1. Graphical representation of Phylogenetic tree constructed from 20 sequences of, Mystriga fragrans, Garcinia gummi-gutta, and Gymnema Sylvestre species Fig 1 shows that Graphical representation of Phylogenetic tree constructed from 20 sequences (Various Medicinal Plants) with the hypothetical root of straight line denoted 100% sequence similarity and branch length denoted non-sequence similarity. Dendrogram is generated using Neighbor-joining method. This results show evolutionary relationship between the medicinal plant species.

### 4. Conclusion

This study is the first of its kind to assess molecular markers based identification and classification of a significant set of medicinal plants. The obtained cladogram based on these three medicinal plants sequence (Mystriga fragrans, Garcinia gummi-gutta, and Gymnema Sylvestre) data is by the use of neighbour-joining method. Comparative analysis of these three medicinal plants are carried out and realized that Gymnema Sylvestre is a plantae group are closely related as treated previously in current taxonomic systems. Further applications

studies will be carry on future perspective associated to *Gymnema Sylvestre*. Almost all parts of the plant have been consumed as food and used In traditional medicine most of plant part is taken as food for the palliation of inflammatory-mediated illness such as gastrointestinal and cardiovascular diseases. The seeds and leaves of plant are reported having antitumor, hypertensive, cardio protective and wound healing properties and are also used for eye diseases. The plant *Gymnema Sylvestre* contains high sequence similarity in blast analysis. Where they are employed in further studies on drug designing, Molecular docking and dynamics and system biological studies in network prediction.

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