CHAPTER 4

DISCUSSION
The Present study revealed a number of both pharmacognostic and phytochemical biomarkers which can be used to distinguish one drug from the other species.

Pharmacognostic characters
Between the two genera *Euphorbia* and *Jatropha*, the latter can be recognized by the presence of rosettes of crystals in chambered cells of the palisade tissues, the crystalloids around the veins of the leaf, and also in primary cortex. Vessel elements with simple perforation plates were common in the genus *Euphorbia* while simple and scalariform perforation plates were found to be present in *Jatropha*. However, there were many variations, which can be seen between the species, which are of diagnostic value.

Within the genus *Euphorbia* paracytic stomata were seen in *E. nerifolia*, *E. nivulia* and *E. tirucalli* whereas others possessed anomocytic type of stomata. The variability in stomatal type among the species investigated confirms with opinions of earlier workers (Metcalfe and Chalk, 1954; Seghal and Paliwal, 1974). Stomatal index and stomatal frequency values can be used to differentiate the genus *euphorbia* since these values are statistically significant. These values were maximum in *E. hirta* 31/mm² and 11.2/mm². Edeoga and Osawe (1996) recognized the significance of stomatal index values in distinguishing between the leaves of closely related Senna species. Similarly vein parameters such as vein islet number and vein termination number also are distinctive. Vein islet number was maximum in *E. thymifolia* (10.7/mm²) even as vein termination number was maximum in *E. geniculata* (37.5/mm²). Levin (1929) emphasised taxonomic value of vein islet areas. He described that vein islet number is nearly constant for species and can be used as a valuable specific character. Hall and Melville (1951; 1954) distinguished between Indian and Alexandrian Senna based on veinlet termination number. Palisade ratio was another feature which also provided an important data for leaf drug evaluation. It was highest in *E. hirta* (8.4) and lowest in *E. microphylla* (2.3).
Trichome morphology also proved to be an important distinguishing feature among the taxa investigated. Similarity in trichome morphology was however, shown by the three *Euphorbia* species. Adaxial short curved trichome proved to be a good distinguishing character of *E. hirta*. Edeoga and Osawe (1996) had noted that diversities in trichome morphology are important taxonomic characters that could be used at both the genus and species levels among different groups of flowering plants.

The transverse section of the midrib revealed that three species of the genus *Euphorbia* examined possessed sheath of green parenchyma cells around the vascular bundles. The results of this study were in line with Seghal and Paliwal (1974) who also reported these structures in *E. hirta* and *E. thymifolia*. The three small bundles in the midrib were a characteristic feature of *E. tirucalli*. Stem also showed variations, collenchyma distribution in the stem was typical. Patches of irregularly thickened collenchyma occur in the hypodermal region of *E. geniculata*. Scattered arrangement of fibres in the cortical region of *E. nerifolia*, *E. nivula* and *E. tirucalli* were remarkable.

Within the genus *Jatropha* paracytic and amphibrachyparacytic stomata occur on both the surfaces and only the abaxial surface. Other variable characters like distribution and density of trichomes among the species investigated substantiate with the previous report of Olowokudejo (1993). The calcium oxalate crystals too showed variations in the size of the average diameter of rosette forms, they were maximum in *J. glandulifera* (63μm) and minimum in *J. podagrica* (21μm). Papillose epidermis and hypostomatic leaves were distinctive in *J. multifida* and *J. podagrica*. Multicellular trichomes on the margins of the leaves were unique to *J. gossypifolia*. The variation of stomatal characters, vein parameters and palisade ratio were also noted within the genus *Jatropha* and were characteristic. Anatomically cortical bundles were notable in the stem of *J. glandulifera*.

Powdered characters were also valuable markers that could go a long way in distinguishing these taxa.

**Phytochemical characters**

The investigated taxa fall into two groups based on the distribution of the type of flavonoids. Among the flavonoids located flavonols were found to be maximum in the
genus *Euphorbia* even as flavones, glycoflavones and proanthocyanidins were found to be maximum in the genus *Jatropha*. Species containing both flavones and flavonols were taken to be intermediate while those having flavones alone were considered relatively advanced. The distribution of leaf flavonoids indicated that the species of *Euphorbia* i.e. *E. geniculata*, *E. hirta*, *E. nivulia* and *E. tirucalli* contained only flavonols and therefore were the most primitive. From the present study the genus *Jatropha* can be considered as advanced. Flavonoids are effective antioxidants because of their free radical scavenging properties, chelators of metal ions and may protect tissues against free oxygen radicals and lipid peroxidation. Quercetin and kaempferol were the flavonols located; both have antihistamine activity and inhibit the release of histamine from immune cells called mast cells, which are involved in the pathogenesis of asthma and allergic reactions (Larson, 1988). Flavonoids and other phenolics have been reported to play a preventive role in the development of cancer and heart diseases.

**The Biomarkers of the plants studied**

1. Pharmacognostic biomarkers.


The plant possesses epidermal papillae (PI) and pole to pole contiguous stomata (Ppc). Both eglandular (Egl) and glandular (Gl) trichomes are present. The eglandular trichomes are uniseriate covering with warty walls having swollen base. The glandular trichome is multicellular and multiseriate capitate with short stalk.

This plant also has eglandular (Egl) and glandular (Gl) trichomes. Eglandular trichomes are two types. 

a. Uniseriate covering with warty walls having oval base. 
b. short curved (Sc) trichome. Glandular (Gl) trichome is multicellular and multiseriate capitate with rounded apex.


This plant possesses groups of stomata (Gr), single guard cell with pore (Sgp) and stomata with cytoplasmic connections (Cy).

The plant possesses amphibrachy paracytic (ABP), parallelocytic (PC) stomata and dumb bell (DB) shaped starch grains.


The plant contains stomata with single subsidiary cell (Ssc) and trilobed (Tl) starch grains.
The plant shows both eglandular and glandular trichomes. Eglandular trichome is uniseriate covering with warty walls having round base (Egl). Glandular trichome is multicellular multiseriate and cylindrical in shape (GI).

This plant contains twin stomata (TS), hemiparacytic (HP) stomata and bone shaped (BS) starch grains.
8. *Jatropha curcas* Linn.

This plant possesses stomata with unequal guard cell (Ugc).


The trichomes observed are eglandular and glandular. Eglandular trichomes are two types.

- a. Unicellular conical
- b. Uniseriate conical trichome. Glandular (Gl) trichome is multicellular and multiseriate capitate with long stalk.
10. *Jatropha gossypifolia* Linn.

This plant contains both eglandular and glandular trichomes. Eglanular trichomes are three types. a. Unicellular covering b. Uniseriate covering. c. Multicellular with conical apex. Glandular trichome (Gl) is multicellular multiseriate capitate with long and cylindrical stalk. The stalk cells are bigger.
11. *Jatropha multifida* Linn.
This plant contains epidermal papillae (Pl) and tetracytic (Tc) stomata.

This plant possesses epidermal papillae (Pl) and actinocytic stomata (AS).
1. Key based on Phytochemical biomarkers
   A. Presence of only flavonols (no flavones).
      1. Quercetin and kaempferol................................. E. hirta.
      2. Quercetin and gentisic acid............................. E. geniculata.
      3. Quercetin and p-OH Benzoic acid....................... E. nivulia.
      4. Quercetin................................................. E. tirucalli.
   B. Presence of only flavones (no flavonols).
      1. Apigenin and acacetin..................................... J. multifida.
      2. Apigenin, acacetin and luteolin....................... J. glandulifera.
      3. Luteolin.................................................. J. podagrica.
      4. Apigenin and luteolin................................... J. gossypifolia.
   C. Presence of both flavones and flavonols.
      1. Apigenin and 3’-OMe quercetin........................... E. microphylla.
      3. Luteolin and kaempferol................................ E. thymifolia.
      4. Apigenin, acacetin and quercetin...................... J. curcas.

2. Key based on both Pharmacognostic and Phytochemical biomarkers.
   A. Presence of both eglandular and glandular trichomes.
      1. Both Flavonols and Flavones.
         1. Apigenin, luteolin and kaempferol..................... E. thymifolia.
      2. Flavonols or Flavones occur singly.
         2. i. Only Flavonols.
            1. Quercetin and gentisic acid.......................... E. geniculata
            2. Quercetin and kaempferol............................ E. hirta.
         2. ii Only Flavones.
            1. Apigenin, Acacetin and luteolin..................... J. glandulifera.
B. Absence of trichome.

1. Both flavonols and flavones.
   1. Apigenin and 3'- OMe quercetin......................................E. microphylla.
   2. Apigenin and kaempferol........................................E. nerifolia.
   3. Apigenin, acacetin and quercetin...............................J. curcas.

2. Flavonols or flavones occur singly.
   2. i Only flavonols
      1. Quercetin and p- OH Benzoic acid............................E. nivulia.
      2. Quercetin..................................................E. tirucalli.
   2. ii Only flavones.
      1. Apigenin and acacetin..................................J. multifida.
      2. Luteolin......................................................J. podagrica.

The biomarkers obtained from the present study can be helpful to distinguish one species from the other making it easier to detect the adulteration in the actual drug plant.