SUMMARY AND CONCLUSIONS

The thesis entitled “PHYTOCHEMICAL AND BIOLOGICAL STUDIES OF SELECTED INDIAN TRADITIONAL MEDICINAL PLANTS, ASPARAGUS RACEMOSUS, AERVA LANATA AND ABRUS PRECATORIUS” embodies the work on the preliminary phytochemical screening and biological studies of the ethanolic extracts and its fractions chloroform and hexane of Asparagus racemosus, Aerva lanata and Abrus precatorius. This has been presented in eight chapters and a chapter wise summary is given below. The summary of the results is mentioned in Table I.

Chapter I
Introduction
An Introduction to plants as a source for drugs, the list of species, some important secondary metabolites which are based as a source of drugs are dealt within the chapter I.

Chapter II
Review of Literature
The morphological characters of the selected plants (Asparagus racemosus leaves, Aerva lanata leaves and Abrus precatorius seeds) with an updated review on phytocompounds and biological activities that have been dealt by eminent scientists in this sphere are taken up in a detailed manner. The aims and objectives of the present study are spelt out there in.

Chapter III
Phytochemical studies
Preliminary phytochemical studies on selected plant extracts and its fractions of Asparagus racemosus, Aerva lanata and Abrus precatorius have been performed for presence of various phytocompounds. The selected plant extracts are also studied for the quantification of total phenols and flavonoid content. The results of this phytochemical studies has been discussed in an elaborate manner.
**Asparagus racemosus**

Qualitative phytochemical screening of ethanolic extract and its fractions chloroform and hexane of *A. racemosus* revealed the presence of different phytochemical constituents i.e. phytosterols, triterpenes, flavonoids, saponins and tannins. All the fractions of *A. racemosus* do not contain alkaloids.

The Quantified phenolic contents of *A. racemosus* extracts were ranging from $5.61 \pm 0.30$ to $10.23 \pm 0.47$ (mg/g). The ethanolic extract have more phenolic content $10.23 \pm 0.47$ (mg/g) than its fractions. The Quantified flavonoid contents was ranging from $11.20 \pm 2.31$ to $20.53 \pm 1.33$ (mg/g). The ethanolic extract has more flavonoid content $20.53 \pm 1.33$ (mg/g) than its fractions.

**Aerva lanata**

Qualitative phytochemical screening of ethanolic extract and its fraction chloroform of *A. lanata* revealed the presence of different phytochemical constituents like phytosterols, triterpenes, flavonoids, saponins, glycosides, tannins, alkaloids and carbohydrates. Hexane fraction does not contain triterpenes and glycosides.

The Quantified phenolic contents of *A. lanata* extracts were ranging from $5.45 \pm 0.45$ to $13.03 \pm 1.07$ (mg/g). The ethanolic extract have more phenolic content $13.03 \pm 1.07$ (mg/g) than its fractions. The quantified flavonoid contents was ranging from $7.87 \pm 0.67$ to $21.20 \pm 1.15$ (mg/g). The ethanolic extract has more flavonoid content $21.20 \pm 1.15$ (mg/g) than its fractions.

**Abrus precatorius**

Qualitative phytochemical screening of ethanolic extract and its fractions hexane and chloroform of *A. precatorius* revealed the presence of different phytochemical constituents like phytosterols, triterpenes, flavonoids, tannins and carbohydrates. All the fractions of *Abrus precatorius* does not contain saponins and glycosides.

The Quantified phenolic contents of *A. precatorius* extracts were ranging from $7.65 \pm 0.62$ to $15.23 \pm 0.60$ (mg/g). The ethanolic extract have more phenolic content $15.23 \pm 0.60$ (mg/g) than its fractions and the flavonoid content was ranging from $6.53 \pm 1.15$ (mg/g) than its fractions.
0.67 to 29.20 ± 1.15 (mg/g). The ethanolic extract has more flavonoid content 29.20 ± 1.15 (mg/g) than its fractions.

Preliminary phytochemical analysis reveals the common phytochemical constituents present among the ethanolic extract of *A. racemosus*, *A. lanata* and *A. precatorius* are phytosterols, triterpenes, flavonoids and tannins.

Quantitative estimation of all the extracts and its fractions reveals that ethanolic extract has more phenolic and flavonoid content than its fractions chloroform and hexane.

The Total phenolic content for the ethanolic extracts of *A. racemosus*, *A. lanata* and *A. precatorius* were in the following order

*A. precatorius* > *A. lanata* > *A. racemosus*

where as the chloroform and hexane fraction were in the following order

*A. precatorius* > *A. racemosus* > *A. lanata*

The Total flavonoid content for the ethanolic extracts of *A. racemosus*, *A. lanata* and *A. precatorius* were in the following order

*A. precatorius* > *A. lanata* > *A. racemosus*

where as the chloroform and hexane fractions were in the following order

*A. racemosus* > *A. lanata* > *A. precatorius*

Chapter IV

*In-vitro* Antioxidant activity

*In-vitro* antioxidant activity on selected plant extracts and its fractions of *Asparagus racemosus*, *Aerva lanata* and *Abrus precatorius* have been performed on different models i.e. Superoxide radical, hydroxyl radical and DPPH radical scavenging activity. All the results of these models have been discussed in an elaborate manner.

**Superoxide radical scavenging activity**

The ethanolic extract and its fractions chloroform and hexane of *Asparagus racemosus*, *Aerva lanata* and *Abrus precatorius* were found to possess
concentration dependent scavenging activity on superoxide radical generated by photoreduction of riboflavin. The mean IC$_{50}$ value of ascorbic acid was found to be 103.22 µg.

The mean IC$_{50}$ values for superoxide radical of ethanolic extract and its fractions chloroform and hexane of $A.\ racemosus$ were found to be 198.64 µg, 350.25 µg and 622.53 µg respectively.

The mean IC$_{50}$ values for superoxide radical of ethanolic extract and its fractions chloroform and hexane of $A.\ lanata$ were found to be 219.65 µg, 299.79 µg and 554.39 µg respectively.

The mean IC$_{50}$ values for superoxide radical of ethanolic extract and its fractions chloroform and hexane of $A.\ precatorius$ were found to be 176.47 µg, 295.21 µg and 576.87 µg respectively.

**Hydroxyl radical scavenging activity**

The ethanolic extract and its fractions chloroform and hexane of $Asparagus\ racemosus$, $Aerva\ lanata$ and $Abrus\ precatorius$ were found to possess concentration dependent scavenging activity on hydroxyl radicals. The mean IC$_{50}$ value of ascorbic acid was found to be 162.17 µg.

The mean IC$_{50}$ values for hydroxyl radical of ethanolic extract and its fractions chloroform and hexane of $A.\ racemosus$ were found to be 244.56 µg, 362.78 µg and 684.54 µg respectively.

The mean IC$_{50}$ values for hydroxyl radical of ethanolic extract and its fractions chloroform and hexane of $A.\ lanata$ were found to be 223.72 µg, 323.39 µg and 614.14 µg respectively.

The mean IC$_{50}$ values for hydroxyl radical of ethanolic extract and its fractions chloroform and hexane of $A.\ precatorius$ were found to be 199.73 µg, 316.08 µg and 596.23 µg respectively.

**DPPH radical scavenging activity**

The ethanolic extract and its fractions chloroform and hexane of $Asparagus\ racemosus$, $Aerva\ lanata$ and $Abrus\ precatorius$ were found to possess
concentration dependent scavenging activity on DPPH radicals. The mean IC\textsubscript{50} value of ascorbic acid was found to be 74.58 µg.

The mean IC\textsubscript{50} values for DPPH radical of ethanolic extract and its fractions chloroform and hexane of \textit{A. racemosus} were found to be 140.64 µg, 251.10 µg and 548.60 µg respectively.

The mean IC\textsubscript{50} values for DPPH radical of ethanolic extract and its fractions chloroform and hexane of \textit{A. lanata} were found to be 168.13 µg, 293.13 µg and 525.46 µg respectively.

The mean IC\textsubscript{50} values for DPPH radical of ethanolic extract and its fractions chloroform and hexane of \textit{A. precatorius} were found to be 135.88 µg, 257.56 µg and 485.53 µg respectively.

Among the ethanolic extracts and its fractions chloroform and hexane of Asparagus racemosus, Aerva lanata and Abrus precatorius better free radical scavenging activity was found in ethanolic extract, the order of activity is in the following manner for superoxide, hydroxyl and DPPH radical.

\textbf{Ethanolic extract > Chloroform fraction > Hexane fraction}

Among the plants, the order of the scavenging activity for superoxide radical and DPPH radical for ethanolic extract is in the following manner.

\textit{A. precatorius} > \textit{A. racemosus} > \textit{A. lanata}

where as for hydroxyl radical is in the following manner:

\textit{A. precatorius} > \textit{A. lanata} > \textit{A. racemosus}

The results of the present study suggest that the tested ethanolic extracts and its fraction chloroform and hexane have antioxidant activity and/or free radical scavenging activity. Literature survey reveals that flavonoids (Lamson and Brignall, 2000; Torres \textit{et al}., 2006) phenolic compounds (Visioli \textit{et al}., 1998; Stratil \textit{et al}., 2006) are responsible for antioxidant activity. Preliminary phytochemical studies of the extracts and its fractions shows the presence of flavonoids and phenolic compounds, therefore the “antioxidant activity might be due to the presence of flavonoids and phenolic compounds in the selected plant extracts”. However, we do
not know what components in the plant extracts/fractions show these activities. More
detailed studies on chemical composition of the plant extracts, as well as other *in-vivo*
assays are essential to characterize them as biological antioxidants.

**Chapter V**

**Toxicity study**

Acute oral toxicity studies in mice of either sex (20-30 g) revealed that the extracts
up to 2000 mg/kg for *Asparagus racemosus* and *Aerva lanata* have not produced
any mortality in experimental animals where as for *Abrus precatorius* the extracts
upto 1000 mg/kg have not produced any mortality in experimental animals.

**Chapter VI**

**Hepatoprotective Activity**

*In-vivo* hepatoprotective activity on selected plant extracts of *Asparagus racemosus*,
*Aerva lanata* and *Abrus precatorius* have been performed on different models i.e.
CCl₄ and paracetamol induced hepatotoxicity in rats. The results of these models
have been discussed in an elaborate manner.

**CCl₄ induced hepatotoxicity**

Effect of ethanolic extracts and its fraction chloroform and hexane of *Asparagus
racemosus, Aerva lanata* and *Abrus precatorius* on percentage protection against
CCl₄ induced hepatotoxicity in rat were calculated and plotted a bar graph. The
serum levels SGPT (270.50 ± 4.79), SGOT (278.83 ± 4.54), ALP (293.17 ± 4.09), TB
(2.93 ± 0.33), CHL (215.67 ± 4.63) was significantly (*p<0.001*) increased and TP
(2.82 ± 0.35), ALB (1.98 ± 0.44) was significantly (*p<0.001*) decreased in CCl₄
treated animals when compared to control. Silymarin (25 mg/kg) treated animals
showed significant (*p<0.001*) decrease in SGPT (67.17 ± 2.33), SGOT (131.67 ±
3.75), ALP (171.33 ± 2.85), TB (1.25 ± 0.19), CHL (111.33 ± 3.96) and significant
(*p<0.001*) increase in TP (6.53 ± 0.38), ALB (4.47 ± 0.33) levels when compared to
CCl₄ alone treated rats.
The ethanolic extract and chloroform fraction of *A. racemosus* at doses of 400 and 800 mg/kg treatment significantly (p<0.001) decreased the levels of SGPT, SGOT, ALP, TB, CHL and significantly (p<0.01) increased the levels of TP when compared to CCl₄ alone treated rats where as the hexane fraction at doses of 800 mg/kg treatment significantly (p<0.001) reversed the levels of SGPT, SGOT, ALP, TB, CHL and significantly (p<0.01) increased the levels of TP when compared to CCl₄ alone treated rats.

The ethanolic extract of *Aerva lanata* doses of 400 and 800 mg/kg treatment significantly (p<0.001) decreased the levels of SGPT, SGOT, ALP, TB and CHL when compared to CCl₄ alone treated rats where as the chloroform and hexane fraction at doses of 800 mg/kg treatment significantly (p<0.001) decreased the levels of SGPT, SGOT, ALP, TB and CHL when compared to CCl₄ alone treated rats.

The ethanolic extract of *Abrus precatorius* at dose of 200 mg/kg treatment significantly (p<0.001) reversed the levels of SGPT, SGOT, ALP, TB, CHL, TP and ALB when compared to CCl₄ alone treated rats where as the chloroform and hexane fractions at doses of 200 mg/kg treatment significantly (p<0.001) reversed the levels of SGPT, SGOT, ALP, CHL and TP when compared to CCl₄ alone treated rats.

The results clearly indicated that the pretreatment with the selected plant extracts and silymarin showed hepatoprotective activity i.e. significant (p<0.001) decrease in all the elevated levels of SGOT, SGPT, ALP, TB and CHL and significant increase (p<0.01) in TP levels when compared to CCl₄ treated group. The activity of the extracts is found to be dose dependant. Among the extracts, better hepatoprotective activity was found in ethanolic extract, the order of activity is in the following manner:

**Ethanolic extract > Chloroform fraction > Hexane fraction.**

The hepatoprotective activity of the ethanolic extracts of *A. racemosus, A. lanata* and *A. precatorius* at maximal dose were in the following order for CCl₄ induced hepatotoxicity

*A. racemosus > A. lanata > A. precatorius*
**Paracetamol induced hepatotoxicity**

Effect of ethanolic extracts and its fractions chloroform and hexane of *Asparagus racemosus*, *Aerva lanata* and *Abrus precatorius* on percentage protection against paracetamol induced hepatotoxicity in rat were calculated and plotted a bar graph. The serum levels SGPT (198.83 ± 0.95), SGOT (248.83 ± 0.87), ALP (297.33 ± 0.71), TB (2.72 ± 0.06), CHL (206.50±1.61) was significantly (p<0.001) increased and TP (2.47 ± 0.26), ALB (1.72 ± 0.16) was significantly (p<0.001) decreased in paracetamol treated animals when compared to control. Silymarin (25 mg/kg) treated animals showed significant (p<0.001) decrease in SGPT (76.67 ± 0.49), SGOT (188.67 ± 1.94), ALP (175.50 ± 0.62), TB (0.73 ± 0.02), CHL (101.67 ± 2.70) and significant (p<0.001) increase in TP (6.37 ± 0.42), ALB (4.47 ± 0.33) levels when compared to paracetamol alone treated rats.

The ethanolic extract and its fractions chloroform and hexane of *Asparagus racemosus* at doses of 400 and 800 mg/kg treatment significantly (p<0.001) decreased the levels of SGPT, SGOT, ALP, TB and CHL when compared to paracetamol alone treated rats.

The ethanolic extract and its fractions chloroform and hexane of *Aerva lanata* at doses of 400 and 800 mg/kg treatment significantly (p<0.001) decreased the levels of SGPT, SGOT, ALP, TB, CHL and significantly (p<0.01) increased the levels of TP and ALB at 800 mg/kg when compared to paracetamol alone treated rats.

The ethanolic extract and its fractions chloroform and hexane of *Abrus precatorius* at doses of 100 and 200 mg/kg treatment significantly (p<0.001) decreased the levels of SGPT, SGOT, ALP, TB and CHL.

The results clearly depicted that paracetamol intoxication in normal rats elevated the serum levels of SGPT, SGOT, ALP, TB and CHL, where as decreased the levels of TP and ALB significantly when compared to control treated group indicating acute hepatocellular damage and biliary obstruction leading to necrosis. The rats pretreated with the selected plant extracts and silymarin showed hepatoprotective activity i.e. significant (p<0.001) decrease in all the elevated levels of SGOT, SGPT,
ALP, TB and CHL and significant increase (p<0.01) in TP levels when compared to paracetamol treated group. The activity of the extracts is found to be dose dependant. Among the extracts, better hepatoprotective activity was found in ethanolic extract, the order of activity is in the following manner:

**Ethanolic extract > Chloroform fraction > Hexane fraction**

The hepatoprotective activity of the the ethanolic extracts of *A. racemosus, A. lanata* and *A. precatorius* at maximal dose were in the following order for paracetamol induced hepatotoxicity.

*A. lanata > A. precatorius > A. racemosus*

Hence, the results of the present study suggests that the tested ethanolic extracts and its fraction chloroform and hexane of *A. racemosus, A. lanata* and *A. precatorius* have hepatoprotective activity against carbon tetrachloride and paracetamol induced hepatotoxicity. Literature survey revealed that flavonoids (Janbaz *et al.*, 2002) and triterpenoids (Oliveira *et al.*, 2005) present in the plants are responsible for their hepatoprotective activity. Preliminary phytochemical analysis of the extracts and its fractions revealed the presence of flavonoids, tannins, triterpenoids and phytosterols in *A. racemosus, A. lanata* and *A. precatorius*, hence the "hepatoprotective activity might be due to the presence of flavonoids and triterpenoids compounds in the selected plant extracts".

The exact bioactive principles responsible for the reduction in elevated serum levels remain to be investigated. Furthermore, it is difficult, at this stage to draw any logical conclusion on the exact mechanism of hepatoprotective activity of the extracts due to such a diverse mixture of phytocomponents contained in the plants of *A. racemosus, A. lanata* and *A. precatorius*.

Biochemical mediators involved in this hepatoprotective process need to be investigated to assess the specific mechanisms of actions of the extracts also to identify the possible lead molecules involved for hepatoprotective activity.
Chapter VII

Anti-inflammatory Activity

*In-vivo* anti-inflammatory activity on selected plant extracts of *Asparagus racemosus*, *Aerva lanata* and *Abrus precatorius* have been performed on carrageenan induced paw oedema in rats and the results of this model has been discussed in an elaborate manner.

The standard drug indomethacin at dose 10 mg/kg significantly inhibited the maximal oedema response by 50.00 ± 4.55 and the total oedema response (AUC) was inhibited by 37.16 ± 2.53 during the 6 h of the carrageenan-induced rat paw acute inflammation when compared to the control group treated with drug vehicle.

The ethanolic extracts and its fractions chloroform and hexane of *Asparagus racemosus* at doses 400 and 800 mg/kg significantly (*p*<0.01) inhibited the maximal oedema response and the total oedema response (AUC) during 6 h when compared to the control group treated with drug vehicle where as the dose of 200 mg/kg significantly (*p*<0.05) inhibited the maximal oedema response and the total oedema response (AUC) during 6 h compared to the control group treated with drug vehicle.

The ethanolic extracts of *Aerva lanata* at doses of 400 and 800 mg/kg significantly (*p*<0.01) inhibited the maximal oedema response and the total oedema response (AUC) during 6 h when compared to the control group treated with drug vehicle where as the chloroform and hexane fraction at a dose of 800 mg/kg significantly (*p*<0.01) inhibited the maximal oedema response and the total oedema response (AUC) during 6 h compared to the control group treated with drug vehicle.

The ethanolic extracts and its fraction chloroform *Abrus precatorius* at doses of 100 and 200 mg/kg significantly (*p*<0.01) inhibited the maximal oedema response and the total oedema response (AUC) during 6 h when compared to the control group treated with drug vehicle where as the hexane fraction at a dose of 200 mg/kg significantly (*p*<0.01) inhibited the maximal oedema response and the total oedema response (AUC) during 6 h compared to the control group treated with drug vehicle.
The activity of the extracts is found to be dose dependant. Ethanolic extracts from the three selected species had produced significant (p<0.01) reduction at the middle and higher doses, where as the chloroform and hexane fractions produced significant (p<0.01) reduction at the maximum dose, hence better anti-inflammatory activity was found in ethanolic extract, the order of activity is in the following manner:

Ethanolic extract > Chloroform fraction > Hexane fraction

The percentage inhibition of the maximal and the total paw oedema during 6 h for the ethanolic extracts of A. racemosus, A. lanata and A. precatorius at maximal dose were in the following order

A. precatorius > A. racemosus > A. lanata

Hence, the results of the present study suggests that the tested ethanolic extracts and its fraction chloroform and hexane of A. racemosus, A. lanata and A. precatorius have anti-inflammatory activity against carrageenan induced paw oedema in rats. Literature survey revealed that flavonoids (Kim et al., 2000) and sterols (Navarro et al., 2001) contents present in the plants are responsible for their anti-inflammatory activity. Preliminary phytochemical analysis of the extracts and its fractions reveals the presence of flavonoids, triterpenoids and phytosterols in A. racemosus, A. lanata and A. precatorius, hence the “anti-inflammatory activity might be due to the presence of flavonoids and sterols compounds in the selected plant extracts”.

Further studies have to be conducted on chronic inflammation to establish the mechanism of action of these extracts that exhibited significant activity and also to identify the exact phytoconstituents involved during the inflammation process.

Chapter VIII

Antimicrobial Activity

Antimicrobial activity of the selected plant extracts of Asparagus raemosus, Aerva lanata and Abrus precatorius have been performed against various Gram +ve bacteria, Gram –ve bacteria and fungi. Antimicrobial screening of the plant extracts
was carried out by the cup plate method. Minimum inhibitory concentration was also carried out.

All the extracts and its fractions chloroform and hexane of *Asparagus racemosus*, *Aerva lanata* and *Abrus precatorius* at a concentration of 100 µg/mL and 300 µg/mL exhibited antibacterial and antifungal activities against one or other organisms in dose dependent manner.

**Asparagus racemosus**

*Asparagus racemosus* ethanolic extract had produced maximum zone of inhibition against *Staphylococcus aureus* (gram +ve), *Escherichia coli* (gram –ve) and *Candida albicans* (fungi) when compared to chloroform and hexane fractions. MIC of ethanolic extract of *Asparagus racemosus* was found to be 12.5 µg/mL against *Bacillus pumilus*, *Staphylococcus aureus* (gram +ve), *Escherichia coli* (gram –ve) and *Candida albicans* (fungi).

**Aerva lanata**

*Aerva lanata* ethanolic extract had produced maximum zone of inhibition against *Staphylococcus aureus* (gram +ve), *Escherichia coli* (gram –ve) and *Candida albicans* (fungi) when compared to chloroform and hexane fractions. MIC of ethanolic extract of *A. lanata* was found to be 12.5 µg/mL against *Staphylococcus aureus* (gram +ve), *Escherichia coli* (gram –ve) and 25.0 µg/mL against *Candida albicans* and *Aspergillus niger* (fungi).

**Abrus precatorius**

*A. precatorius* ethanolic extract had produced maximum zone of inhibition against *Staphylococcus aureus* (gram +ve), *Escherichia coli* (gram –ve) and *Candida albicans* (fungi) when compared to chloroform and hexane fractions. MIC of ethanolic extract of *A. precatorius* was found to be 6.250 µg/mL against *Bacillus pumilus*, *Escherichia coli* (gram –ve) and *Candida albicans* (fungi).

Among the selected three plant extracts and its fractions, the ethanolic extracts had produced good antimicrobial activity at 100 and 300 µg/mL dose, than chloroform and hexane fractions. The chloroform and hexane fractions produced antimicrobial
activity at 300 µg/mL. The minimum inhibitory concentration among the extracts of the selected three species was in the following order

**Ethanolic extract > Chloroform fraction > Hexane fraction**

From the results of the minimum inhibitory concentration (MIC) presented; it was observed that the broadest activity of the extract was against *E. coli*, *S. aureus*, *B. pumilus* and *C. albicans*.

The antimicrobial activity of the ethanolic extracts of *A. racemosus*, *A. lanata* and *A. precatorius* was found to be dose dependent and were in the following order

**A. precatorius > A. racemosus > A. lanata**

Hence, the results of the present study suggests that the tested ethanolic extracts and its fraction chloroform and hexane of *A. racemosus*, *A. lanata* and *A. precatorius* have antimicrobial activity. Literature survey reveals that the sterols (Pereira et al., 2006), flavonoids (Rauha, 2000) and phenolic compounds (Proestos, 2005) show anti microbial activity against several pathogens (Eloff, 2004). Preliminary phytochemical analysis of the plant extracts and its fractions shows the presence of sterols, flavonoids and phenolic compounds in *A. racemosus*, *A. lanata* and *A. precatorius*. Hence, the broad-spectrum “antimicrobial activity exhibited by the ethanolic extract and its fraction chloroform could be related with the concentrations of sterols, flavonoids and phenolic compounds present in these selected plant extracts”.

The results obtained in producing antimicrobial activity support the folkloric claims regarding the plants and their medicinal values. It is therefore, suggested the isolation and possible characterization of the active constituent(s) from the extracts of this plant species as possible antimicrobial agents.
Table I: Summary of the results

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<th>Plant</th>
<th>Extract / fractions</th>
<th>Phytochemical</th>
<th>In-vitro Anti oxidant activity</th>
<th>Hepatoprotective activity</th>
<th>Anti-inflammatory activity</th>
<th>Anti microbial activity</th>
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+++: High  ++: Moderate  +: Less