Chapter-V

Results and Discussion

*Boswellia serrata* and *Meusa ferrea* is an important medicinal plant in Indian traditional medicine. Both medicinal plants were frequently used in *Ayurveda* and *Allopathy* (Table-4 and 5). The main aim of this study was, therefore, to carry out preliminary phytochemical screening and antimicrobial activities of Gum Resin of *Boswellia serrata* and flower buds extracts of *Meusa ferreae*. (Table-10 and 16)

The concern of this study was the chemical investigations of Gum Resin of *Boswellia serrata* performed on different types of solvent for extraction. Preliminary phytochemical screening of the plant revealed the presence of polyphenols, saponins, terpenoids and phytosterols. (Table-10)

Isolated compound-I, Gum Resin of *Boswellia serrata* (*Family: Burseraceae*) identified as 3-*α*-hydroxy-*lup-20* (29)-*en-3β*-ol ;(lupeoll) (lupeol). The investigations on acid fractions of 10% Ba(OH)$_2$ *Boswellia serrata* by TLC bands were observed at R$_f$ 0.43 (Table-12), on further purification with EtOAc:Hexane and characterized as below:
Physical State: Yellow Crystal Solid

Melting Point: 215-216\(^0\)C

Molecular Formula: C\(_{30}\)H\(_{50}\)O\(_4\)

Molecular Weight: 427.3754

UV \(\lambda_{\text{max}}\) (MeOH) at 203.5 nm 254 nm

IR: 3426 cm\(^{-1}\) (66) and 3404 cm\(^{-1}\) (86) -OH stretching, 3071 cm\(^{-1}\) (60) -CH stretching in methylene, 2943 cm\(^{-1}\) (04) -CH stretching in methyl, 2870 cm\(^{-1}\) (28)-CH stretching in symmetrical cyclic alkene,
2868 cm\(^{-1}\) (28)-CH stretching in terminal methyl, 1644 cm\(^{-1}\) (60) unconjugated C=C stretching in alkene, 1458 cm\(^{-1}\) (37) -CH bending in 6 or 7 member ring, 1381(31) -OH stretching, 1372 cm\(^{-1}\) (55) -CH bending in methyl, 1082 cm\(^{-1}\) (72) C-O stretching in alcohol, 964 cm\(^{-1}\) (77) -CH out of plane bending in R\(_2\)C=CH\(_2\), 882 cm\(^{-1}\) (55) -CH bending in R\(_2\)C=CH\(_2\), 870 cm\(^{-1}\) (58) -CH bending in R\(_2\)C=CH\(_2\).

![Chemical Structure](image)

\(\text{3-}\alpha\text{-hydroxy-lup-20-(29)-en-3\beta-ol}\)
$^1$H-NMR spectra measured in chloroform at 300.1 MHz with the chemical shift $\delta$ 1.01, 0.93 ppm (3H,s,H-24), 0.92 ppm (3H,s,H-27), 0.83 ppm (3H,s,H-23), 0.82 ppm (3H,s,H-26) and 0.78 ppm (3H,s,H-25), 4.66 and 4.54 (2H, $d$, 2, 2, H-29), with low frequency coupling constant ($J = 2.4$Hz).

Isolated compound-II, Gum Resin of *Boswellia serrata* (*Family: Burseraceae*) identified as 3-$\beta$-acetoxy-12-ursen-28-oic acid was isolated from 95% ethanolic extract.

The investigations on acid fractions of 10% Ba(OH)$_2$ *Boswellia serrata* by TLC bands were observed at $R_f$ 0.51.

(Table-12), characterized as below:

Physical State: Light Yellow Crystal Solid

Melting Point: 258-260$^0$C

Molecular Formula: C$_{30}$H$_{50}$O$_4$

Molecular Weight: 456

UV $\lambda$max (MeOH) at 206 nm

IR: 2961 cm$^{-1}$ (16) -CH stretching in $-\text{COCH}_3$ , 2925 cm$^{-1}$ (04) -CH stretching in $-\text{COCH}_2$ , 2855 cm$^{-1}$ (20) -CH stretching in $\text{CH}_3$, 1710 cm$^{-1}$ (57) $>\text{C}=\text{O}$
stretching in –COOH ,1468 cm⁻¹(47) -CH bending in CH₂ ,1447 cm⁻¹(52) -CH bending in –CH₃,1377 cm⁻¹ (49) -CH bending in –CH₃,1276 cm⁻¹ (37) -OH in plane bending in –COOH ,1199 cm⁻¹ (79) -C-O stretching in –COOH ,1141 cm⁻¹ (72) -CH bending in –CH₂,1005 cm⁻¹ (74) 6 or 7 membered ring stretching ,985 cm⁻¹ (70) C-H out of plane bending in trans RCH=CHR,972 cm⁻¹ (79) -CH out of plane bending RCH=CH₂ ,965 cm⁻¹ (72) -CH out of plane bending trans RCH=CHR, 907 cm⁻¹ (81) -O-H out of plane bending, 826 cm⁻¹ (86)-C-H out of plane bending β-cyclic ring ,724(84) =CH out of plane bending, 662 cm⁻¹ (79) -CH out of plane bending RCH=CHR.

3-β-Acetoxy-12-Ursen-28-Oic Acid
\(^1\text{H-NMR spectra measured in chloroform at 400 MHz}\) with the chemical shift 0.74 (3H, s, H-19), 0.82 (3H, s, H-28), 0.87 (3H, s, H-30), 0.93 (3H, s, H-18), 0.99 (3H, s, H-29), 1.11 (1H, dd, 11.8, 2.0, H-5), 1.2 (2H, dd, 9.9, 3.1, H-1), 1.37 (2H, dd, 12.2, 6.8, H-16), 1.53 (2H, dd, 14.4, 7.3, H-7), 1.58 (3H, s, H-27), 1.67 (3H, s, H-26), 1.75 (2H, dd, 12.8, 3.0,H-12), 2.27 (1H, dd, 12.1, 3.5, H-17), and 3.23 (1H, dd, 11.5, 4.4, H-3) \(\delta\) 1.70 ppm.

The chemical investigations of flower buds extracts of Meusa ferreae performed on different types of solvent for extraction. Preliminary phytochemical screening of the plant revealed the presence of polyphenols, flavonoids, coumarins, saponins, and phytosterols. (Table-16)

Isolated compound-I, flower buds of Meusa ferrea Linn. (Family: Clusiaceae) identified as 3’, 5, 7-trihydroxy-4-methoxyflavone-7–rutinoside. It was extracted with 95% ethanol. The concentrated ethanolic extract was successively extracted with the ethyl acetate soluble fraction was subjected to concentration under reduced pressure and on TLC examination over (n-Hexane: EtOEt: HCOOH 75:20:5 and iodine vapours and visualizing agent), showed brown bands at \(R_f\) 0.54. (Table-18)

It was characterized as: Physical State: light yellow crystalline solid (soluble in MeOH)
Melting Point: 268-269°C

Molecular Formula: C₁₆H₁₂O₆

Molecular Weight: 300

IR: 3634 cm⁻¹ (60) -OH stretching (free) in 2° alcohol, 3470 cm⁻¹ (52) -OH stretching (bonded/steric hindered), 3410 cm⁻¹ (52) -OH stretching in phenol, 2964 cm⁻¹ (16) -CH₃ a symmetric stretching -O CH₃, 2924 cm⁻¹ (04) -CH stretching in CH₃ (two bands), 2869 cm⁻¹ (25) -CH stretching in CH₃ (two bands), 2866 cm⁻¹ (16) -CH₃ symmetric stretching -O CH₃, 1662 cm⁻¹ (47) -CH₃ out of plane bending vibration (overton and combination band), 1611 cm⁻¹ (37) chealed >C=O, 1591 cm⁻¹ (57) aromatic ring, 1466 cm⁻¹ (36) -C-H methylene scissoring, 1424 cm⁻¹ (66) -CH₂ scissoring in RCH=CH₂, 1377 cm⁻¹ (50) -CH in plane symmetric bending in –CH₃, 1305 (57) -CH₂ rocking in RCH=CH₂, 1203 cm⁻¹ (57) C-O stretching and O-H in plane bending (coupled) in phenol, 1098 cm⁻¹ (50) -OH bending and C-O stretching of primary alcohol, 1070 cm⁻¹ (43) -CH₂ rocking in RCH=CH₂, 1066 cm⁻¹ (60) ex-C-O stretching in ring, 822 cm⁻¹ (66) C-C stretching vibration.
Compound-II, identified as **5,7-dihydroxy-3-(p-hydroxyphenyl)-4-oxo-4H-chromene** isolated from flower buds of *Meusa ferrea* Linn. (*Family: Clusiaceae*) was extracted with 95% ethanol. The concentrated ethanolic extract was successively extracted with the ethyl acetate soluble fraction was subjected to concentration under reduced pressure and on TLC examination over (n-Hexane: EtOEt: HCOOH 75:20:5 and iodine vapours and visualizing agent), showed brown bands at R$_f$ 0.62 (Table-18).
It was characterized as: Physical State: light yellow crystalline solid (soluble in MeOH)

Melting Point: 335-336 °C

Molecular Formula: C_{15}H_{10}O_{5}

Molecular Weight: 270

IR: 3414 cm\(^{-1}\) (22) -OH stretching in polymeric alcohol, 3143 cm\(^{-1}\) (36) intramolecular H-bonded with >C=O, 3133 cm\(^{-1}\) (36) intramolecular H-bonded with >C=O, 3117 cm\(^{-1}\) (36) -CH\(_2\) asymmetric stretching, 3037 cm\(^{-1}\) (46) -CH\(_2\) symmetric stretching, 1652 cm\(^{-1}\) (04) C=O stretching in unsaturated ketone, 1616 cm\(^{-1}\) (12) C=C stretching in polyene, 1583 cm\(^{-1}\) (32) C=C stretching in Ph-CH=CHR, 1426 cm\(^{-1}\) (38) =C-H\(_2\) scissoring in unsaturated alkene, 1318 cm\(^{-1}\) (20) >C=O in cyclic ketone, 1055 cm\(^{-1}\) (55) C-O-C stretching in cyclic Ether, 852 cm\(^{-1}\) (66) C-H out of plane bending in aromatic ring, 820 cm\(^{-1}\) (43) C-H bending in aromatic ring.

Compound-III, 7-Isopropoxy-3-Phenyl-4H-1-Benzopyran-4-one (Ipriflavone) isolated from flower buds of *Meusa ferrea* Linn. (*Family: Clusiaceae*) was extracted with 95% ethanol. The concentrated ethanolic extract was successively extracted with the ethyl acetate soluble fraction was
subjected to concentration under reduced pressure and on TLC examination over (n-Hexane: EtOEt: HCOOH 75:20:5 and iodine vapours and visualizing agent), showed light brown bands at $R_f$ 0.78 (Table-18). The above concentrated light brown mass was crystallized from chloroform: methanol (1:1). It was characterized as: Physical State: brownish crystalline solid

Melting Point: 114-115°C

Molecular Formula: $C_{18}H_{16}O_3$

Molecular Weight: 280.32

Iprilflavone

IR: 3094 cm$^{-1}$ (81) >C=C< stretching in aromatic ring, 2925 cm$^{-1}$ (04)-C-H stretching in alkane, 2854 cm$^{-1}$ (10)-C-H stretching in alkane, 1637 cm$^{-1}$ (44) >C=O stretching with conjugation, 1602 cm$^{-1}$ (67) >C=C< stretching in ether vinyl, 1564 cm$^{-1}$ (80) aromatic 1497 cm$^{-1}$ (76) alkane, 1377 cm$^{-1}$ (35) -CH bending in -CH(CH$_3$), 1093 cm$^{-1}$ (76) -C-O stretching in branched aliphatic, 1026 cm$^{-1}$ (82) -CH in plane bending in aromatic ring, 933 cm$^{-1}$
(81) -CH bending in -CH(CH₃), 694 cm⁻¹ (61) -CH out of plane bending in aromatic ring.

Antimicrobial activities in vitro of the aqueous and 95% ethanolic crude extracts Gum Resin of Boswellia serrata and flower buds extracts of Meusa ferreae were investigated against gram +ve and gram -ve bacteria by agar disc diffusion method.

To find out the growth of micro-organism, we had used two types of inhabitants like Antibiotic (Ampicillin and Penicillin) and ethanolic extracts of two different medicinal plants of different family Boswellia serrata (Family: Burseraceae) and Meusa ferrea (Family: Clusiaceae), their comparative results for antibacterial activity was discussed as per available in recorded data.(Table-23 & 24)

When Boswellia serrata and Meusa ferrea was used as 25µg/ml then it was observed that micro-organism like Bacillus pumilus, Bacillus mycoids, E.coli, Klebsiella sps., Salmonella sps., Staphylococcus albas, Staphylococcus aureus and Shigella sps. had minimum/nil zone of inhibition while with increase in concentration of active ethanolic extracts the zone of inhibition increases it was maximum i.e. 4 mm with the higher concentration i.e.75 µg/ml and 100 µg/ml in the interaction of Bacillus
mycoids, Klebsiella sps., and Proteus sps. under influence of Boswellia serrata and Meusa ferrea.

The air dried gum resin powdered coarsely 100 mesh of Boswellia serrata and Meusa ferrea was extracted with 95% ethanolic and the residues were recovered using a evaporations. the ethanolic extracts was further subjected to the broth micro dilution method to determine MIC. The maximum activity was observed against bacteria namely Bacillus mycoids and Proteus sps. with the 100 µg/ml. While in concern of Meusa ferrea with 100 µg/ml, Klebsiella sps. and Proteus sps. were more effective (Table-25 & 26). Effect of antibiotics like ampicilin, penicillin were also studied qualitatively (Table 25 & 26).

Bacillus pumilus and Klebsiella sps. had no response regarding its antibacterial activity when it was brought into contact of 25µg/ml, 50µg/ml contents of each Boswellia serrats and Meusa ferrea respectively. (Table -24)

The ethanolic extracts of Boswellia serrata and Mesua ferrea were subjected to know their antifungal activities over Candida albicans, Rhizopus, Mucor and Aspergillus fumigatus qualitatively using different concentration in the same study Griseofulvin was effective against all these fungus on the same concentration.