INVESTIGATION OF PHYTOCHEMICALS FROM METHANOLIC EXTRACT OF STEM BARK OF COMMIPHORA MUKUL

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Abstract

Lawsaritol (A), Episesamin (B), Sesamin (C), Quercetin (D) and Ferulic acid (E) were obtained from a methanol extract of the stem bark of Commiphora mukul (Burseraceae). By using 1H, 13C NMR, IR, and MS spectroscopy as part of elemental analysis, the structures of the isolated compounds were verified.

Keywords: Commiphora Wightii, Guggul, Resin, Phytochemicals, In Vitro, Antioxidant Activity, Ascorbic Acid.

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1. INTRODUCTION

Commiphora mukul (Syn: Commiphora wightii), also known as "Indian bdellium," "Guggulu," or "Guggul" in India, is a highly endangered plant that is significant for medicine and is a member of the Burseraceae family[1]. C. mukul is found in the Aravalli hills (in India), particularly in Gujarat and Rajasthan. It is dioecious, highly branched, and grows to a height of 4 metres. Its branches are knotty, twisted, and spirally ascending, with sharp spines at the ends of each one. Guggul was chemically examined and found to contain a variety of substances, including lignans, lipids, terpenoids, flavonoids, sugars, amino acids, and steroids[2,3]. Flowers include d-glucuronide, ellagic acid, and pelargonidin. Seed oil[3] contains linoleic acid, palmitic acid, steric acid, campesterol, cholesterol, and stigmasterol.

Guggulipid, an extract of this gum, is used as a medication in Ayurveda and Unani to treat obesity[3]. Gugulipid derived from this plant significantly decreased the lifespan of the human prostate cancer cell line LNCaP (androgen-dependent) and its androgen-independent version with an IC50 of one millimicron (twenty four hour treatment) and suggested a potential involvement in apoptosis and cancer prevention[2]. Nodulocystic acne (Skin disease) has been successfully treated with gugulipid administration[2]. This plant's isolated guggulosterone has the power to increase the catabolism of low density lipid cholesterol (LDL) and promote the binding of LDL receptors in hepatocytes, as well as to prevent LDL cholesterol's oxidative modification[4]. The active components of resin, guggulsterone-Z and -E, are what provide resin its lipid-lowering effects on human blood[4]. In streptozotocin-induced diabetic mice, alcoholic C. mukul gum-resin extract has anti-inflammatory and hyperglycemic properties[5].

2. MATERIAL AND METHOD

Experimental Procedures:
Melting Point of compound/s was determine by melting points apparatus. IR spectra were recorded on FTIR SHIMADZU 8400S spectrometer by using KBr. Using TMS as the internal standard, the 1H-NMR and 13C-NMR spectra were captured in $CDCl_3$ at 300 MHz and 75 MHz, respectively. Argon/Xenon was used as the FAB gas, and spectra were captured using a JEOL SX 102/DA-6000 mass spectrometer.

Plant Material:
With the assistance of the local villagers, the stem bark of C. mukul was harvested in Bikaner, Rajasthan (India).

Extraction and isolation of the compounds:
1500 gm of stem bark of plant material was extracted in MeOH for 48 hours. Crude extract was produced when obtained extract was concentrated below abridged force. The methanolic extract was converted into slurry by being dissolved in the least quantity of MeOH and adsorbed on silica gel. Column chromatography was performed on the dried slurry over silica gel. The succeeding compounds i.e. A - E were isolated, purified, and characterised after being eluted from the column using various solvents in order of escalating polarity.

Isolation of compound A as Lawsaritol:
Petroleum ether and benzene were used to elute a column in a 1:2 ratio, and the compound-A was obtained, and it produced a white solid upon evaporation. It was crystallised from ethanol, and 123–124°C was determined to be its melting point. After spraying 10% H_2SO_4 and heating to 100^0C, TLC revealed the typical triterpene pink colour. The blue green hue produced by the Liebermann-Burchard reagent indicated the substance was a sterol. Unsaturation was indicated by the decolorization of Bayers reagent. IR (KBr, cm^{-1}): 3440 (-OH, str.), 1615 (C=C, str.), 1055 (C-O, str.), 1390, 1385 and 1320. 1H NMR ($CDCl_3$, δppm): 3.49 (m, 1H, C3), 5.42 (d, 1H, C4), 0.64 (s, 3H, C18), 0.97 (s, 3H, C19), 1.22 (d, 3H, C21), 0.86 (d, 3H, C26), 0.92 (d, 3H, C27), 0.95 (t, 3H, C29), 1.83 (m, 1H, C25), 1.44-1.92 (m, for enduring 28 protons). ^{13}C NMR ($CDCl_3$, δppm): 37.28 (C1), 31.65 (C2), 70.65 (C3), 120.50 (C4), 145.10 (C5), 22.10 (C6), 34.65 (C7), 30.82 (C8), 49.15 (C9), 35.52 (C10), 22.10 (C11), 41.25 (C12), 43.25 (C13), 57.80 (C14), 26.30 C15), 29.20 (C16), 55.25 (C17), 12.97 (C18), 20.10 (C19), 35.85 (C20), 19.75 (C21), 40.15

(C22), 25.90 (C23), 46.25 (C24), 30.10 (C25), 20.15 (C26), 18.95 (C27), 24.65 (C28) and 12.90 (C29). MS (m/z): 414 (M^+) etc. Molecular formula $C_{29}H_{50}O$.

Isolation of Compound B as Episesamin:

By eluting the column with 100% C_6H_6 compound B was obtained. Acetone was used to crystallise the finished product, producing white, tiny crystals. Melting point was displayed as 121–22^{O}C. IR (KBr, cm^{-1}): 2855 (C–H str.), 1525 (>C = C< str.), 1255, 1080, 1045 (C–O-C str.). ^{1}H NMR ($CDCl_3$, δppm): 3.25 (m, 1H, C1), 4.78 (d, 1H, C2), 4.12 (d, 1H, C4), 3.85 (m,1H, C4), 2.90 (m, 1H, C5), 4.35 (d, 1H, C6), 3.30 (m, 1H, C8), 3.81 (m, 1H, C8), 5.90 (s, 4H, -OCH_2O-), 6.80-6.95 (m, 6H, aromatic protons). ^{13}C NMR ($CDCl_3$, δppm): 50.45 (C1), 81.90 (C2), 70.90 (C4), 53.80 (C5), 86.95 (C6), 70.65 (C8), 131.20 (C1′), 136.14 (C1′′), 106.40 (C2′), 107.10 (C2′′), 146.65 (C3′, C3′′), 148.10 (C4′, C4′′), 109.20 (C5′, C5′′), 117.65 (C6′), 118.95 (C6′′), 99.90 (-OCH_2O-). MS (m/z): 354 (M^+). Molecular formula $C_{20}H_{18}O_6$.

Isolation of Compound C as Sesamin:

Sesamin was produced by eluting the column with C_6H_6 and $CHCl_3$ (3:1), which was then refined from acetone to produce a chemical with a cream colour. IR (KBr, cm^{-1}): 2860 (C–H str.), 1505 (>C = C< str.), 1245, 1065, 1035 (C–O–C stre.). ^{1}H NMR ($CDCl_3$, δppm): 3.10 (s, 2H, C1, C5), 4.65 (d, 2H, C2, C6), 3.82 (dd, $2H_{ax}$, C4, C8), 4.15 (m, $2H_{eq}$, C4, C8), 6.75-6.90 (m, 6H, aromatic protons), 5.81 (s, 4H, -OCH_2O-). ^{13}C NMR ($CDCl_3$, δppm) : 55.60 (C1, C5), 87.10 (C2, C6), 73.05 (C4, C8), 136.55 (C1′, C1′′), 107.10 (C2′, C2′′), 146.10 (C3′, C3′′), 147.80 (C4′, C4′′), 108.60 (C5′, C5′′), 120.75 (C6′, C6′′), 102.40 (–OCH_2O–) MS (m/z): 354 (M^+). Molecular formula $C_{20}H_{18}O_6$.

Isolation of compound D as Quercetin:

Compound D was extracted by eluting the column with $CHCl_3$ and EtOAc in equal ratio. After the solvent was eliminated, the resultant substance was crystallised with MeOH and CH_3COCH_3 in a equal ratio into light yellow needles having a melting point of 315–316°C. It results in a positive ferric chloride test, which shows the presence of flavonoids when the compound's alcoholic solution is combined with a few drops of a neutral ferric chloride solution. IR (KBr, cm^{-1}): 3465 (-OH str.), 3020 (Ar., C–H str.), 1595, 1535 (Ar., C-C str.), 915, 820, 785. ^{1}H NMR ($CDCl_3$, δppm): 12.32 (s, 1H, -OH at C5), 10.25 (s, 1H, -OH at C3), 8.85 (s, 1H, -OH at C7), 8.87 (s, 1H, -OH at C3'), 8.42 (s, 1H, -OH at C4'), 7.22 (m, 2H, C2′ and C6′), 7.10 (d, 1H, C5′), 6.32 (d, 2H, C6 and C8). ^{13}C NMR ($CDCl_3$, δppm): 155.92 (C2), 133.86 (C3), 177.28 (C4), 161.82 (C5), 99.32 (C6), 165.75 (C7), 94.50 (C8), 157.10 (C9), 105.76 (C10), 124.91 (C1′), 113.55 (C2′), 144.35 (C3′), 148.60 (C4′), 114.34 (C5′), 121.83 (C6′). MS (m/z): 302 (M^+), 284, 152, 105, 95 etc. Molecular formula $C_{15}H_{10}O_7$.

Isolation of compound E as Ferulic acid:

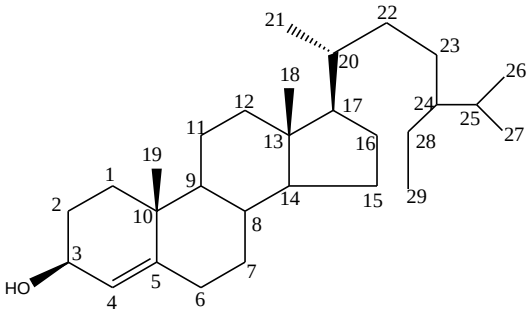
When ethyl acetate was used to elute the column, light yellow crystals were produced. Melting point was displayed as 168–169°C. IR (KBr, cm^{-1}): 3455, 1695, 1610, 1515, 1280, 945. ^{1}H NMR ($CDCl_3$, δppm): 3.94 (s, 3H, H4'), 6.34 (d, 1H, J = 15 Hz, C2'), 6.93 (d, 1H, J = 9.0 Hz, C6), 7.22 (dd, 1H, J = 8.0 and 2.0 Hz, C5), 7.08 (d, 1H, J = 2.0 Hz, C3), 7.78 (d, 1H, J = 15 Hz, C1'). ^{13}C NMR ($CDCl_3$, δppm): 54.98 (C4'), 108.48 (C5), 113.39 (C2), 113.78 (C2'), 124.57 (C3), 127.68 (C4), 147.81 (C1'), 148.05 (C6), 149.37 (C1), 172.36 (C3'). MS (m/z): 179, 161, 133, 105, 89, 77, 51. Molecular formula $C_{10}H_{10}O_4$.

3. RESULT AND DISCUSSION

Characterization of compound A as Lawsaritol:

The compound's mass spectrum revealed m+ at m/z=414. The bands owing to the hydroxyl group (3440 cm^{-1}, broad), C=C group (1615 cm^{-1}), and gem dimethyl groups were visible in the IR absorption spectra (1390, 1385 and 1320 cm^{-1}). The distinguishing peaks in compound A proton-NMR spectra ($CDCl_3$, δppm) were a doublet observed at 5.42 that corresponded to hydrogen at C4 and a multiplet at 3.49 that corresponded to axial hydrogen at C3 interacting amid C2 axial, C2 equatorial, and protons at C4 position. A double bond among C4 and C5 in ring was suggested by the downfield shift of the C3 proton at 3.53 in compared to the C3 proton of β-sitosterol and the appearance of the olefinic proton as a doublet at 5.42. Lawsaritol-identical peaks in the spectra were also

observed. In 13C-NMR spectrum ($CDCl_3$, δppm) signals at δ 145.10, 120.50 and 70.65 corresponded to the unsaturated carbons C5, C4 and C3 respectively. Other signals were appeared at 37.28 (C1), 31.65 (C2), 22.10 (C6), 34.65 (C7), 30.82 (C8), 49.15 (C9), 35.52 (C10), 22.10 (C11), 41.25 (C12), 43.25 (C13), 57.80 (C14), 26.30 C15), 29.20 (C16), 55.25 (C17), 12.97 (C18), 20.10 (C19), 35.85 (C20), 19.75 (C21), 40.15 (C22), 25.90 (C23), 46.25 (C24), 30.10 (C25), 20.15 (C26), 18.95 (C27), 24.65 (C28) and 12.90 (C29). Other signals were also found matching with those previously reported[6,7] for Lawsaritol. Compound-A was characterized as Lawsaritol on the source of above spectral studies.

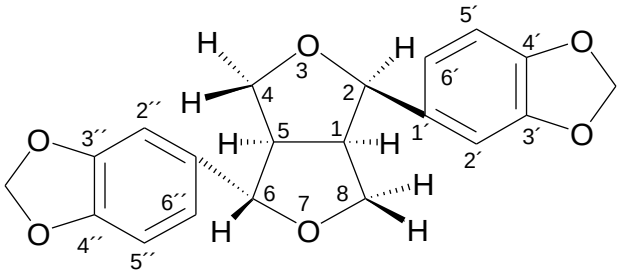


Compound A

Characterization of compound B as Episesamin:

The molecular ion peak in the mass spectrum was found at m/z 354 (M+). The presence of twenty carbon atoms and eighteen protons was confirmed using 13CNMR and 1HNMR respectively. Thus, $C_{20}H_{18}O_6$ was determined to be compound B as molecular formula. The C-H stretching was noticed around 2855 cm^{-1} on the FTIR spectrum. For C-O-C stretching, the absorptions at 1255, 1080, and 1045 cm^{-1} were assigned. A 1525 cm^{-1} absorption band verified the presence of aromatic ring stretching by >C=C.

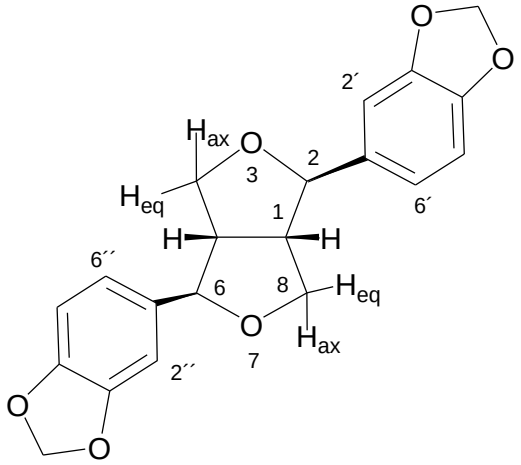
This compound's proton NMR spectrum ($CDCl_3$, δppm) revealed a multiplet from 6.80 to 6.95 that corresponded to six protons of an aromatic ring. At 5.90, a singlet of two methylenedioxy group protons (-OCH2O-) was seen. A proton in C2 position was assigned a doublet at 4.78 (J = 4.95 Hz). At 4.35 (J = 7.32 Hz), the proton in C6 position was confirmed as a doublet. At the C4 location, oxymethylene protons were detected as a multiplet at 3.85 for one proton and a doublet at 4.12 (J = 9.33 Hz) for two protons. For each proton present at C8 location, two multiplets at 3.30 and 3.81 were allocated. Two protons were present and were established for the C1 and C5 protons at 3.25 and 2.90, respectively. The absorptions seen at 50.45 and 53.80 in the 13C NMR spectra ($CDCl_3$, δppm) of this compound were ascribed to the C1 and C5 carbons, respectively. The absorption at 99.90 verified two methylenedioxy groups were present. The existence of aromatic carbons were assigned by the absorptions at 131.20 (C1'), 136.14 (C1"), 106.40 (C2'), 107.10 (C2"), 146.65 (C3', C3"), 148.10 (C4', C4"), 109.20 (C5', C5"), 117.65 (C6'), 118.95 (C6") and their assignment have been shown in parentheses. Other signals to be found at 81.90 (C2), 70.90 (C4), 86.95 (C6), and 70.65 (C8) were recognized accordingly. The above 1H NMR and 13C NMR spectral data of compound B were found alike to episesamin[8,9,10]. Compound B was recognised as episesamin based on the description above and the spectrum information.



Compound B

Characterization of compound C as Sesamin:

The number of protons in the molecule was estimated at eighteen using 1H NMR, while 13C NMR confirmed the existence of 20 carbon atoms in the compound. As a result, the chemical formula for component C was determined to be $C_{20}H_{18}O_6$ on the basis of the findings mentioned above. The typical absorption at 2860 cm^{-1} in the FTIR spectra indicates the existence of C-H stretching. The existence of aromatic C=C stretching was indicated by an absorption band at 1505 cm^{-1}. Other significant peaks for C-O-C stretching were seen at 1245, 1065, and 1035 cm^{-1} in addition to this absorption. The presence of 06 aromatic protons was established by seeing a multiplet from 6.75 to 6.90 in the proton NMR spectra (CDCl3, δppm). 04 protons from two methylenedioxy groups were assigned to a sharp singlet seen at 5.81. A doublet at 4.65 (J = 6.09 Hz) indicated the presence of two protons at locations C2 and C6. A multiplet at 4.15 proved the presence of two axial protons at C4 and C8 positions in an equatorial configuration, while the double doublet at 3.82 (J = 13.70, 4.89 Hz) indicated the presence of the protons there. A sharp singlet at 3.10 for two protons was seen for the protons connected at sites C1 and C5. In the 13C NMR spectrum ($CDCl_3$, δppm) of compound 4, absorptions at 55.60 was assigned for C1 and C5 carbon atoms. The methylenedioxy (-OCH_2O-) carbon showed absorption at 102.40. The presence of twelve aromatic carbons were observed at 136.55 (C1', C1"), 107.10 (C2', C2"), 146.10 (C3', C3"), 147.80 (C4', C4"), 108.60 (C5', C5") and 120.75 (C6', C6") and their assignment have been given in parenthesis. In 2,6-diaryl-3,7-dioxiabicyclooctane ring the signals were observed at 87.10 for C2 and C6 and 73.05 for C4 and C8 carbon atoms respectively. On the basis of these observations and previously reported data[8,11-13] compound was identified as sesamin.

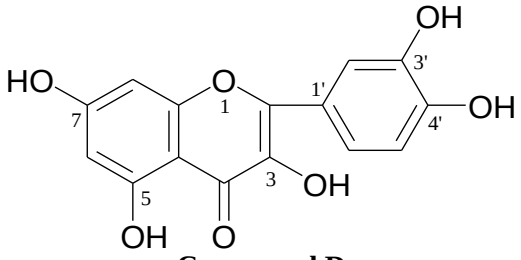


Compound C

Characterization of compound D as Quercetin:

Mass spectrum analysis at 302 [M+] was used to determine the chemical formula of compound-D, which is $C_{15}H_{10}O_7$. A broad peak at 3465 in the IR (KBr, cm^{-1}) spectrum revealed the presence of the hydroxyl group. Along with the aromatic C=C stretching at 1595 and 1535, the aromatic C-H stretching was seen at 3020. The meta-coupled protons at

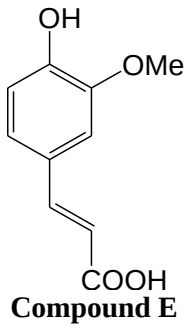
C6 and C8 locations, respectively, showed a set of doublets at 6.32 (J = 2.5 Hz) in the 1H-NMR spectra ($CDCl_3$, δppm). The proton at C5' location was given credit for a doublet seen at 7.10 (J = 8.4 Hz). At 7.22, the protons at the locations of C2' and C6' were seen as overlapping doublets (J = 2.5 Hz) and quartets (J = 2.5, 8.4 Hz), respectively. 05 hydroxyl groups connected at locations C5, C3, C7, C3', and C4' were identified as the source of five singlets seen at 12.32, 10.25, 8.85, 8.87, and 8.42. The absorption at 177.28 (C4) in the 13C-NMR spectra of ($CDCl_3$, δppm) revealed the presence of one carbonyl group, and these values were allocated based on previously reported values[8,14]. The hydroxyl group-attached carbon atoms C3, C5, C7, C3', and C4' displayed absorptions at 133.86, 161.82, 165.75, 144.35, and 148.60, respectively. At 155.92 (C2), 99.32 (C6), 94.50 (C8), 157.10 (C9), 105.76 (C10), 124.91 (C1'), 113.55 (C2'), 114.34 (C5'), and 121.83 (C6'), other absorptions were also noted. Quercetin was identified as compound D based on the aforementioned information.



Compound D

Characterization of compound E as Ferulic acid:

The molecular ion peak for the known phenolic molecule, ferulic acid, with the molecular formula $C_{10}H_{10}O_4$, was seen in the EI-MS at m/z 194 (M+, base peak). The IR spectra verifies the structure of ferulic acid with peaks at 3455 cm^{-1} (-OH, str.), 1695 cm^{-1} (C=O, str.), 1280 cm^{-1} (C-O, str.), and 1515; 1610 cm^{-1} (Ar., C=C). The methoxy group's distinctive signal was visible in the 1HNMR spectrum at 3.94(s). Three aromatic proton resonances at 6.93 (d, J = 9.0 Hz), 7.22 (dd, J = 8.0 and 2.0 Hz), and 7.08 (d, J = 2.0 Hz) in the compound spectrum were also visible. These resonances correspond to the C6, C5, and C3 of the aromatic component of the isolated molecule. Additional two proton doublets with J = 15 Hz were found at 6.34 and 7.78, respectively, which pointed to the presence of C2' and C1' in the compound's side chains. According to the suggested structure of ferulic acid[14,15-17] (4-hydroxy-3-methoxycinnamic acid), the 13CNMR spectra revealed the existence of 10 signals, of which 6 were from aromatic carbon and 4 were from aliphatic chains.



Compound E

4. CONCLUSION

Many Natural chemical molecules have been isolate from plant and they are sources for new drug designing. The investigation was therefore carried out to isolate and describe the active ingredients of the medicinally significant and extensively utilised plants for the healthy life of the livings, inspired by these great achievements in the field of plant chemistry.

Conflicts of Interest: The authors declared no conflict of interest.

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